JOURNAL OF DAIRY SCIENCE

SEPTEMBER, 1956

In this Issue:

Milk Flavor Uniformity Is Possible

Making the Most of Dairy Farm Records

A Letter to the Editor

Dairy People in the News and Important Industry Events

Fifteen Articles Dealing with Results of Studies in the Field of Dairy Science

Abstracts of Literature

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PEOPLE and EVENTS
in the Dairy Science World

All-Industry Entertainment Set for Fall Dairy Meetings

A four-evening program of all-industry entertainments is now well along in the planning stage for this year's fall conventions of Intern. Assoc. of Ice Cream Manufacturers and Milk Industry Foundation, and the 20th Dairy Industries Exhibition, sponsored by Dairy Industries Supply Assoc.

These three major groups will convene in Atlantic City, N.J., the week of Oct. 29–Nov. 3. In addition to the three named associations, the National Assoc. of Retail Ice Cream Mfrs.; Dairy Industries Society, International; and Evaporated Milk Assoc. will meet in Atlantic City during the same week, as will numerous other industry-connected groups.

To provide wholesome and interesting industry entertainment for all dairy industry conventioneers, whether they are members of any of the groups meeting or not, the three associations, IAICM, MIF and DISA, began in 1954 to sponsor jointly all-industry entertainment features in connection with the autumn gatherings. This cooperation continues in 1956, with a large committee of Atlantic City area volunteers from all three organizations now completing arrangements. Planned so far are:

Oct. 28—"Salute to Canada!" an evening reception, with a famous musical group furnishing entertainment.

Oct. 30—"Dairy Rally and Awards Night," a banquet saluting state and regional dairy products associations and climaxing with presentation of awards to winners of the Collegiate Students' Intern. Contest in Judging Dairy Products.

Oct. 31—"Theatre Night in Atlantic City," a one-time-only musical review produced expressly for dairy industry guests in Atlantic City. As in 1954, baritone Earl Wrightson will serve as master of ceremonies; top-flight variety acts and a world-famous comedian are currently also being sought for the performance.

Nov. 2—"Dairy Clambake," an informal evening in one of Atlantic City's major seafood restaurants where guests may select from a variety of sea food menus, with no planned program.

In addition, special ladies' events, such as fashion teas, sight-seeing trips, and boat rides, are also planned for wives of dairy industry visitors.

Honorary D.Sc. to "Bill" Petersen

W. E. PETERSEN received an honorary Doctor of Science degree, honoris causa, from Univ. of Vermont. The following citation was read by President CARL W. BORGMANN at the June commencement exercises:

"Because of your outstanding contributions to better dairy practices throughout the world, because of your devotion to high ideals in teaching and in scientific research and because of your ability to bring the results of this research in everyday language to your fellow man, we delight to honor you."

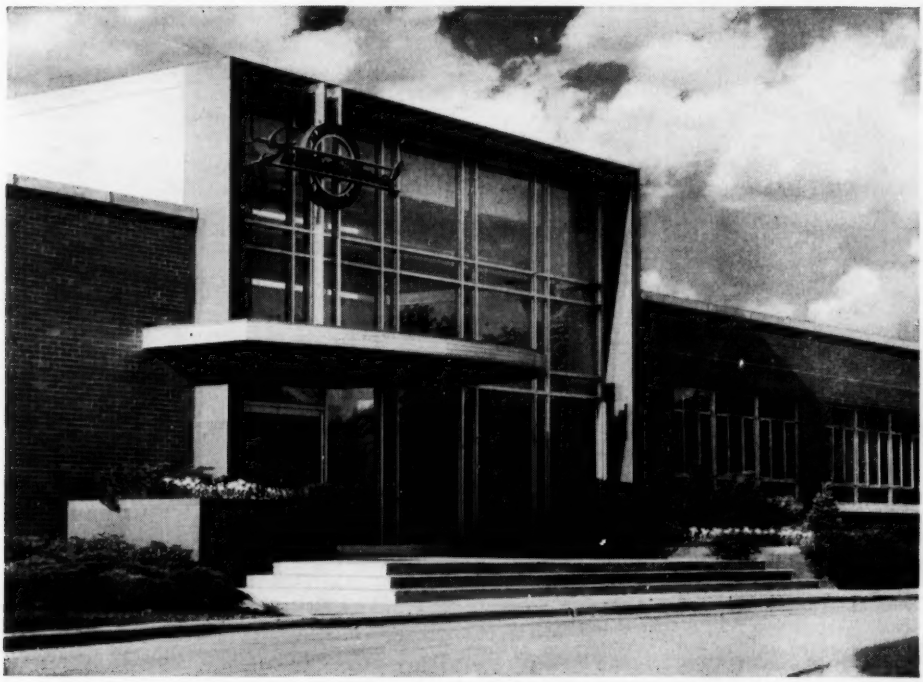
Independent Yankees Finally Agree to Cooperate

According to a Memorandum Agreement signed by the presidents and board of trustees of their respective institutions last June, the Univ. of Vermont and the Univ. of Maine will coordinate and complement their undergraduate programs in dairy manufacturing. It is understood that all dairy manufacturing students who are classified as resident students of the state of Maine and who are in good standing at the Univ. of Maine at the end of their second year have the privilege of completing their program at the Univ. of Vermont. To this end, the Univ. of Maine will develop a 2-year program in dairy manufacturing, and the Univ. of Vermont will continue its 4-year degree program in this field.

Opportunities in Foreign Service

DSI, as cooperator with the Foreign Agricultural Service of the USDA in developing and supervising market development programs for dairy products in other countries, has so far assigned 15 volunteers from the U. S dairy industries to work abroad. The Society is now assembling a roster of other qualified persons from the U. S. dairy industries who may be available for the following types of assignment:

1. Owners or responsible executives of dairy enterprises whose abilities and experience qualify them to head small, hard-working Market Frontier teams; these men must be able to deal effectively with U. S. governmental personnel and foreign governmental, industrial, agricultural, commercial, health and educational personnel for
   (*a*) 4 weeks to 8 weeks market potential, market development procedures, sur-



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Samuel Brody Dies

SAMUEL BRODY, professor of dairy husbandry and a member of the Univ. of Missouri staff, 1920-1956, died of a heart attack while at work Aug. 6, 1956. Words cannot describe fully the greatness of his scientific contributions. His was one of the greatest minds engaged in basic research and applied science in agriculture and the livestock industry, but his contributions covered many fields beneficial to human welfare.

He was an authority on growth, aging, metabolism, productive processes, and environmental physiology of domestic animals. He was the recipient of many honors, and his work brought world-wide recognition to himself and to the University of Missouri. He won a Guggenheim Foundation Fellowship for study in Europe, 1930-31; was the recipient of the Borden Award for research in dairy production, 1950, and the National Distinguished Service Gamma Sigma Delta Award, 1955. Special grants in support of his work, chief among which were funds provided by the National Research Council, Herman Frasch Foundation, United States Navy and the Atomic Energy Commission, totaled more than $250,000.

Brody joined the Dairy Husbandry staff of the Univ. of Missouri in 1920 as assistant professor, became an associate professor in 1925, and was advanced to the rank of professor in 1945. He also carried similar ranks in Agricultural Chemistry. He served as chairman of the Herman Frasch Foundation for Research in Agricultural Chemistry (Dairy Husbandry Project) Missouri Agricultural Experiment Station 1929-40; as chairman of the Station Project on muscular work in farm animals since 1941, and as chairman of the committee on influence of climatic factors on physiological reactions and productivity in farm animals since 1946.

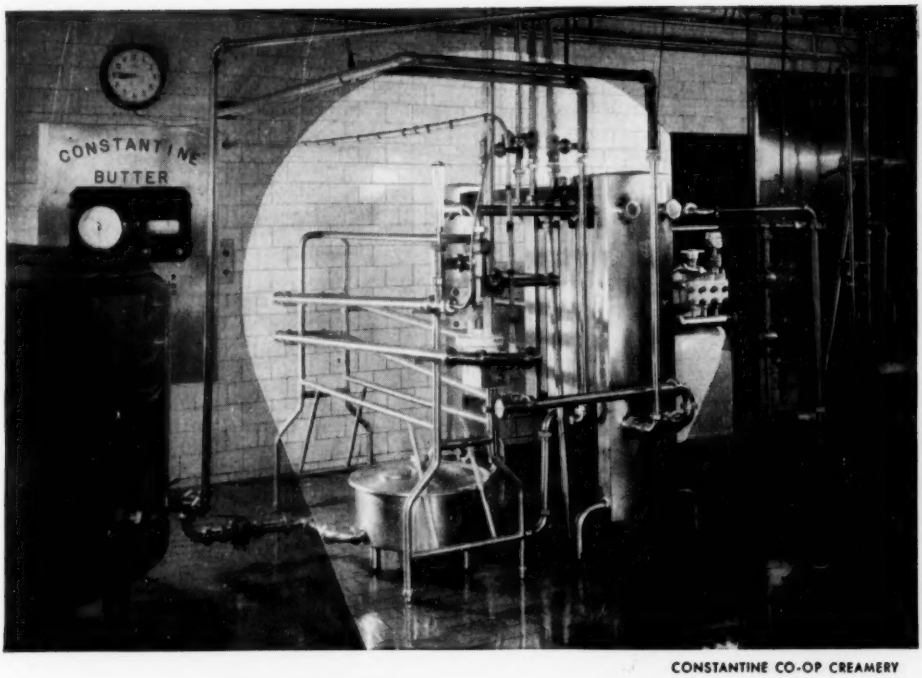
Dr. Brody was a Fellow, American Association for the Advancement of Science; member of the American Society of Naturalists, National Research Council (member of the subcommittee on energy metabolism in relation to agriculture, subcommittee on standardization), American Association of University Professors, American Chemical Society (member, Division of Biological Chemistry), American Society of Biological Chemists, American Institute of Nutrition, American Dairy Science Association, American Society of Animal Production, Missouri Historical Society, Missouri Academy of Sciences, Czechoslovak Academy of Agriculture, Sigma Xi, Gamma Sigma Delta. Clubs: University, Philosophical (Columbia, Missouri). Author: *Bioenergetics and Growth*, 1945; also chapters in several books and encyclopedias, numerous scientific papers and 100 research and station bulletins of the Missouri Agricultural Experiment Station. Degrees: A.B., Univ. of California, 1917; A.M., 1919; Ph.D., Univ. of Chicago, 1928.

Samuel Brody was born in Garbatchi, near Baranowitz, Lithuania (now Poland), Feb. 8, 1890, the son of Avram J. and S. Deborah (Malov) Brody. He came to the United States in 1906 and became naturalized in 1912. He served with the Air Service, also with the Development Division of the Chemical War Service, U. S. Army, 1918-19. He was assistant biochemist, Univ. of California Medical School, 1919-20. He married Sophie Edith Dubosky, August 14, 1920. There are two children—Dr. Eugene Bloor Brody and Dr. Arnold Jason Brody.

veys abroad (no compensation; expenses paid outside U. S. A.);

(*b*) 12 to 24 month assignments as Market Frontier Mission heads abroad (suitable compensation).

2. Dairy technologists of sound practical training and experience in dairy herd and dairy farm management; or in varied types of dairy processing; or in dairy sanitation extension work for
   (*a*) 4 weeks to 8 weeks survey or demonstration assignments abroad (no compensation; expenses paid outside U. S. A.);
   (*b*) 12 to 24 months educational and demonstration assignments abroad (suitable compensation).
3. Consumer relations men; merchandise promotion men; or men with a blend of both these backgrounds and skills; experienced with milk and dairy products for assignments similar to those under 2.
4. Dairy dietary education and demonstration specialists for assignments comparable to those under *2* and *3*.
5. Dairy enterprise executives; dairy technicians; public relations specialists; dietary specialists for



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Staff Changes at Arizona

J. B. FITCH, who recently retired as head of the Dairy Division at the Univ. of Minnesota, became head of the Dept. of Dairy Science at Tucson, Sept. 1.

F. G. HARLAND, who has done dairy production work in the department since 1946, has resigned to operate a dairy farm near Adrian, Mich.

R. G. FOSSLAND of the Univ. of Nebraska joined the Arizona staff Sept. 1.

Staphylococcus Food Poisoning

E. G. REUTER, Yamhill County Health Officer, Oregon, has reported a small outbreak of food poisoning, which occurred after the ingestion of sandwiches containing ham and cheese. The incubation period varied from 2 to 5 hours. Laboratory examination of specimens of a precooked ham revealed the presence of large numbers of hemolytic *Staphylococcus aureus;* and a few of the same organisms were found in a specimen of a pasteurized process cheese. The cheese may have been contaminated by a knife used to slice the ham. No lesions were found on the hands of food handlers.

Grassland Farming

Section O. of AAAS has arranged a rather comprehensive program on the theme: "Grasslands in our National Life" for the New York meeting Dec. 26-31. This program has three major parts:

I. Symposium of invitation speakers, Dec. 29-30.

II. Companion programs on grasslands by several individual societies.

III. Volunteer papers by any qualified person on Grassland Reports — Research and Practice, Dec. 27 and 28.

According to H. B. SPRAGUE of Pennsylvania State Univ., chairman, members of A.D.S.A. are eligible to submit volunteer papers. Titles of such papers should be sent immediately to Mr. Sprague.

This program is designed to provide an opportunity for workers in many scientific disciplines to consider points of view and research on grassland problems by workers in other fields. It is hoped that this exchange of information will provide a broader understanding of this national resource than is normally obtained by attending meetings of a single society.

New York Celebrates Founding of First Butter Factory

The first butter factory in the United States was founded by W. R. WOODHULL, at Campbell Hall, N. Y., in 1856. A ceremony commemorating the centennial anniversary of this important event will take place in the park of the Presbyterian church, Campbell Hall, on Oct. 26, at 11 o'clock. Dinner will be served.

A state historic marker that points to the spot where the creamery stood has been planted on state highway 207. Near the spring, the site of the butter factory, a boulder monument will be set, which will be marked with a plaque bearing the following words:

This SPRING, with an abundance of
cool water, determined the site of the
first butter factory in the United States

1856

R. W. WOODHULL, Owner

GEORGE GOUGE, Buttermaker

— —

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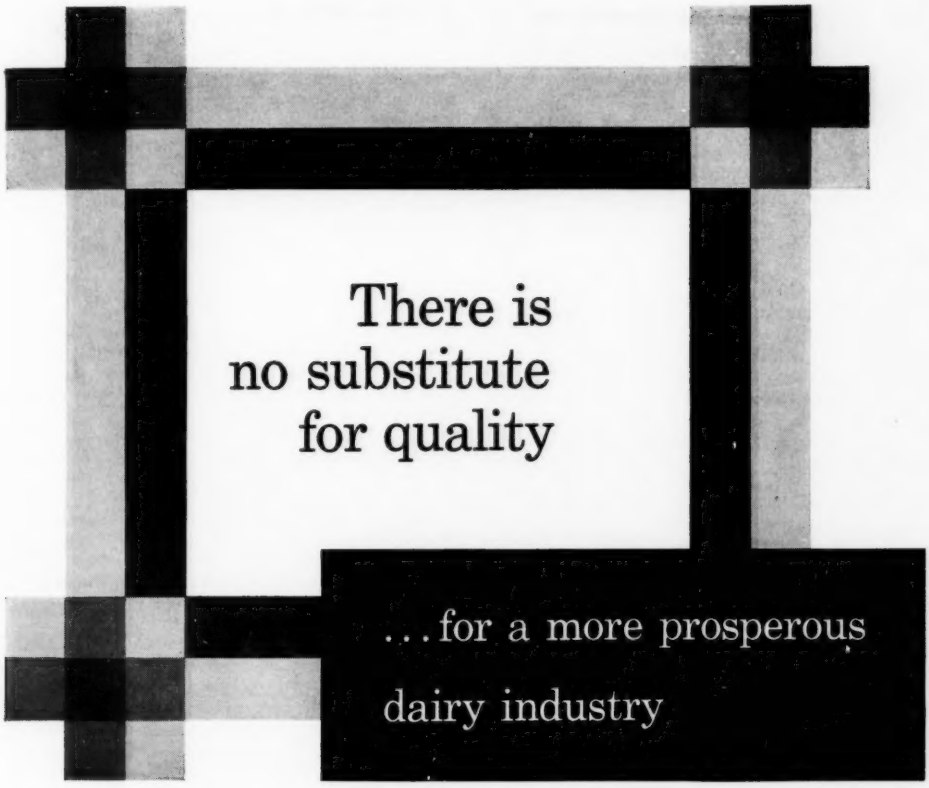
1956

Georgia Departments Reorganize

According to a newsletter recently released, the Board of Regents of the Univ. of Georgia has approved a plan whereby the Departments of Animal Husbandry and Dairy Husbandry will be combined into a new Division of Animal Industry. H. B. HENDERSON will retain the title of department head.



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Engineering Conference

The fifth annual National Dairy Engineering Conference will be held at Michigan State Univ. Feb. 26 and 27, 1957. The meeting is sponsored by the Department of Agricultural Engineering in cooperation with the Departments of Dairy and Continuing Education.

Ohio State Announces Series of Events

N. G. FAWCETT has been appointed the eighth president of The Ohio State Univ., succeeding HOWARD BEVIS. Dr. Fawcett has been superintendent of the Columbus school system and is known as a thorough and efficient administrator. He received a B.S. degree in 1931 from Kenyon College and a Master's degree from Ohio State in 1937 and was awarded an honorary Doctor of Law degree from Kenyon in 1952.

I. A. GOULD was a member of the United States delegation to the XIVth Intern. Dairy Congress in Rome, Sept. 24-28. Dr. Gould served as vice-chairman of the section on economics and technical problems.

The Employer Appreciation Luncheon is planned for Oct. 8. The luncheon, followed by a football game, is an annual event and is the students' way of expressing thanks to their summer employers.

The Eighth Annual Dairy Technology Homecoming Brunch is scheduled for Oct. 27.

Dairy technology students are again planning to attend the Dairy Exposition at Atlantic City, Oct. 29–Nov. 3. The University will furnish transportation with the students paying the rest of their expenses.

The Sixth Annual Market Milk Short Course will be held Nov. 5-16. This course, designed for employees in plant, sales or management, offers studies on processing, elementary bacteriology and chemistry, quality control, product evaluation, cultured products, and milk plant equipment.

The Ohio Dairy Products Assoc., in conjunction with the Dept. of Dairy Technology, has scheduled a series of conferences for fieldmen to be held this fall. The purpose of the conference is to improve the effectiveness of the fieldmen in producing better quality milk.

North Carolina News

G. S. PARSONS has joined the Dairy Extension staff to fill the vacancy created upon the retirement of F. R. FARNHAM. After receiving his B.S. and M.S. degrees at West Virginia Univ., Parsons was a member of the Dairy Department at the Univ. of Tennessee for 3 years, after which he worked as a fieldman for the Pet Milk Co. in eastern Tennessee. After serving 41 years as extension dairy specialist, Farnham retired to take on the duties of assistant manager for Yadkin Valley Dairy Coop., Wilkesboro, N. C.

J. E. LEGATES has recently been honored by being appointed to the William Neal Reynolds Distinguished Professorship in Dairy Cattle Breeding. The Distinguished Professorship was made possible by an endowment contributed by the late William Neal Reynolds of the Reynolds Tobacco Co. It is the highest honor bestowed upon the professors in the School of Agriculture who have distinguished themselves in research, scholarship, and performance. Nine of these professorships have been awarded in the School of Agriculture in recent years. Among the recipients is GEORGE H. WISE, head, Nutrition Section, N. C. State College.

Hult Appointed Delegate to International Dairy Congress

MILTON HULT, president of the National Dairy Council, was nominated by Secretary of Agriculture, EZRA TAFT BENSON, to be a member of the official U. S. delegation to the Intern. Dairy Congress, which was held in Rome, Sept. 24-28.

As a delegate, Hult was invited to serve as a section discussion leader. He initiated discussion on the nutritional importance of milk in the human diet and promotional methods for increasing milk consumption. He also served as a judge in the Intern. Contest of Paintings for Advertising Posters on Sept. 21, and during his stay in Rome he was in charge of an exhibit displaying NDC and ADA promotional materials.

Virginia Convention to Be Held in January

The 42nd Annual Convention of the Virginia Dairy Products Assoc. will be held at the Hotel Roanoke, Roanoke, Va., Jan. 21 and 22, 1957.

Officers of the association are: president, D. H. CRAUN, Southern Dairies Inc., Norfolk; vice-president, H. H. SCOTT, Valley of Virginia Coop. Milk Producers Assoc., Harrisonburg; vice-president, F. K. LORD, JR., Richmond Dairy Co., Richmond; and secretary-treasurer, C. L. FLESHMAN, Lynchburg-Westover Dairy, Lynchburg.

Glenn Joins Kentucky Staff

WILBURN E. GLENN has been appointed assistant professor and assistant dairy technologist in the Dairy Section of the Univ. of Kentucky. The position to be filled by Dr. Glenn is a new post. He will do research in dairy manufacturing and will help fill the vacancy in the staff caused by the 2-year leave of absence granted H. B. MORRISON in June, 1955, to help establish a dairy plant in Cochabamba, Bolivia.

Dr. Glenn received his B.S. degree in dairy science at Oklahoma A. and M. College in 1948

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October 29—November 3 **Atlantic City, N. J.**

and was listed as the outstanding student of his class. He received his M.S. degree from the same institution in 1952. The Ph.D. degree was granted in 1955 by Washington State College.

After completion of his undergraduate work, Dr. Glenn worked as superintendent in several dairy plants in Kansas and Arizona. He joined the Kentucky department Sept. 1 after serving on the staff of the Puget Sound Milk Market Administrator.

Study to Be Made of Bulk Handling

A 3-year nation-wide research study on bulk handling of milk from the farm to the processor, to be conducted jointly by Oregon State College, Iowa State College, and Cornell Univ., was announced recently by MACK GEBERT, secretary-treasurer of Dairy Products Marketing Assoc., Inc., which will finance the project.

"The study is intended to go into all phases and ramifications of this rapidly growing dairy industry development," stated Mr. Gebert, "and we are hopeful that the findings will be of benefit to both producers and consumers. Among the many factors into which inquiry will be made are (*a*) the frequency of pick-up of milk from the farm; (*b*) quality of dairy products; (*c*) the economic impact of the bulk handling method on the dairy farmer and future marketing patterns."

College representatives attending the initial conference were P. M. BRANDT, head of the Department of Dairying, Oregon State College; F. E. NELSON, professor of dairy bacteriology, Iowa State College; and LELAND SPENCER, professor of marketing, Cornell Univ. RICHARD HOYT, Champaign, Ill., will be coordinator of the project.

Mention

Journal of Dairy Science

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Eastern Section of A.D.S.A. Meets

The eastern section of the American Dairy Science Association met at West Springfield, Mass., Sept. 17.

Kentucky to Hold November Conference

The Fourth Annual Dairy Manufacturing Short Course of the Univ. of Kentucky will be held at Lexington, Nov. 27-29, according to T.R. FREEMAN, professor in charge of dairy manufacturing. The course is being sponsored jointly by the University's Dairy Section and the Dairy Products Assoc. of Kentucky. The three days of the conference will be devoted to a study and discussion of problems of ice cream, market milk, and cheese, respectively. A short course banquet will be held on Nov. 28.

A feature of the meeting will be a panel discussion of the various types of vacuum-treatment operations now available. This discussion will be concerned with practical day-to-day operations, as well as benefits as they affect consumption of milk. A very popular feature, the product clinics, will be a part of the program also. An ice cream clinic will be held on Nov. 28, to be followed by a cultured products clinic on the 28th and a cheese clinic on the 29th.

Completed Thesis

M.S. Degree:

R. E. STAPLES—The extension and freezing of bovine semen. Univ. of Saskatchewan, Saskatoon, Canada.

Consumer Data

In a consumer analysis made in 13 Illinois markets during 1956—

82.3% of all families reported the use of margarine

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LETTERS TO THE EDITOR

In recent years there has been an increasing number of different methods proposed for the measurement of free fatty acids in milk and other dairy products. The results of all of these methods have been reported in terms of acid degrees (mL N base/100 g. fat), regardless of the efficiency of the various methods in recovering quantitatively the total free fatty acids present in the products. With the recent increase of interest in rancidity and lipase, there has also been an increased interest in the various methods available. As a result, confusion within the industry and occasionally even between research workers in various institutions has arisen in regard to the meaning of the acid degree values obtained by the different procedures.

Basically, four different methods are currently in use for following the activity of milk lipase. All of these methods have a definite place in our industry; but, certainly, clarification is needed in terms of interpretation of different researchers' results. The four methods generally in use include titration of churned fat, titration of fat extracted by the USDA detergent procedure, titration of fat extracted by a mixed ether and alcohol extraction procedure, and titration after chloroform butanol silica gel extraction. The average acid degree values and the acid degree value for detection of rancidity vary in each procedure. The data in Table 1 reveal the range of values reported by various workers in fresh milk, the average acid degree value in fresh milk, the threshold value for detection of rancidity and the relative recovery of the free fatty acids. Definite disparity in the results obtained by the different methods is indicated, which can be related directly to the efficiency of the particular procedure in recovering the water soluble free fatty acids.

In order to avoid confusion in interpretation of results, the method used should be clearly defined in all publications concerning acid degree values. It is suggested that free fatty acids measurements reported as acid degree values include a reference to the method used. For the purpose of clarity, the citation should be complete enough to fully identify the procedure. For example, a citation might read "acid degree (Johnson & Gould, *J. Dairy Sci.*, *32*: 447. 1946)." This could be shortened in subsequent reference in the same article to "acid degree (J&G)."

W. JAMES HARPER
Department of Dairy Technology
Ohio State University
Columbus

AND

B. L. HERRINGTON
Department of Dairy Industry
Cornell University
Ithaca, New York

TABLE 1

Method	Acid degree reported for normal milk		Acid degree value for detection of rancidity[a]	Approximate % recovery of water soluble acids
	Range	Average		
1. Churning and titration of fat	0.3-1.0	0.7	1.5	10
2. Detergent (USDA) extraction and titration of fat	0.2-0.9	0.6	1.3	none
3. Solvent extraction and titration of fat	0.4-1.5	0.9	2.0	40
4. Silica gel–solvent extraction and titration	0.4-1.7	1.0	2.1	95

[a] These are approximate values only; much variation should be expected.

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* In view of numerous requests, a reprint has been issued of the review article in Vol. 23, No. 3 (1955) by D. H. L. Rollinson, "Hereditary factors affecting reproductive efficiency in cattle," priced 2s, 6d. ($0.35).

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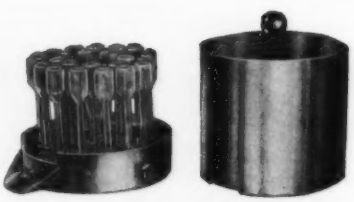

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THE ION-EXCHANGEABLE CALCIUM IN RAW AND HEATED SKIMMILK AS DETERMINED BY THE "RESIN CONTACT TIME METHOD"[1]

J. M. BAKER[2] AND C. W. GEHRKE

Department of Agricultural Chemistry, University of Missouri, Columbia

Calcium, magnesium, phosphate, and citrate ions have an important relationship to the stability of the colloidal systems in milk. An ion-exchange resin contact time method was used to study the equilibria of calcium in composited herd milk. Protein stability was determined by a modification of the Storrs test. The effect on the exchangeability of the calcium of heating skimmilk to 40°, 60°, and 80° C. for 30 minutes was determined. *Editor.*

The stability of the colloidal systems in milk has been a subject of much consideration by investigators in dairy chemistry, especially in the production of concentrated milk products. The factors that determine the stability of the colloidal proteins and salts are considered by many to be physico-chemical in nature. Gel formation, salt precipitation, and protein destabilization are commonly observed in stored milk products such as condensed and evaporated milks. Calcium, magnesium, phosphate, and citrate ions are considered as important ionic constituents in the over-all stability problem. As a consequence, a number of different experimental approaches have been used in an attempt to clarify the chemistry and the phasic distribution of the inorganic components in milk and processed milks and their relationship to the observed defects.

The methods which have been used in the study of these problems include dialysis by Mattick and Hallett *(10)* and Lampitt *et al. (8)*; ultrafiltration by Weingärtner *(15)*; protein precipitation techniques of Magee and Harvey *(9)*, Pyne *(11)*, and Verma *(14)*; and ultracentrifugation by Chanutin *et al. (2)*. These procedures require extensive apparatus and/or long time intervals to complete the experiments, during which time the inorganic equilibria may markedly change.

Magee and Harvey *(9)*, Mattick and Hallett *(10)*, and Jenness *(6)* have studied the effect of various processing conditions on the diffusibility through collodion membranes of calcium and phosphorus in milk and milk products. Mattick and Hallett reported that heating milk to temperatures of 51.7 to 68.3° C. for 30 minutes reduced the diffusibility of calcium. Daum *(3)* heated milk at

Received for publication November 10, 1955.

[1] Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 1566. Approved by the Director.

[2] The experimental data in this paper were taken in part from a dissertation presented by J. M. Baker to the Graduate School, University of Missouri, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1955. Present address: Tennessee Polytechnic Institute, Cookeville.

106° to 110° C. for 6 minutes and to 100° C. for 30 minutes and found that the diffusible calcium decreased upon standing after heating. In later work Hilgemann and Jenness *(5)* observed a significant decrease in the ultrafilterable calcium and phosphorus in milks heated to 67°, 78°, and 87° C. for 30 minutes. When these heated milks were allowed to stand for 48 hours at 5° C. the ultrafilterable calcium and phosphorus in the heated aged milks approached the same values as those of the unheated milk. These data indicated a reversibility in the inorganic equilibria of calcium and phosphorus which were initially changed by the heating process. These differences in reported results may arise as a consequence of the different experimental techniques employed. The colloidal inorganic and protein systems that occur in milk and which may be altered during heat treatments are probably subject to the condition of the collodion membranes used in dialysis and ultrafiltration experiments.

The major objective of this investigation was to study the effect of heat on the equilibria of calcium in skimmilk.

EXPERIMENTAL METHODS

An ion exchange resin contact time method (RCTM) developed earlier *(1)* was employed to study the equilibria of calcium in milk and to measure directly the ion-exchangeable calcium. The resin Amberlite IRC-50 *(12)* was used and initially placed in the potassium form with a 5% solution of potassium hydroxide. Then the resin was buffered to a pH of 6.8 with a buffer mixture of potassium hydroxide and potassium dihydrogen phosphate. Twenty-five milliliter samples of skimmilk were contacted with a measured quantity of the ion-exchange resin (15 or 30 ml. of damp resin) for contact times over the range of 0 to 300 seconds. After contact, the resin was immediately washed free of sample with double distilled water, and the calcium ions retained by the resin were eluted with 1:3 hydrochloric acid. The eluates were made to volume and analyzed by a modification of the ethylenediaminetetraacetate titration procedure *(4, 7)*. All pH measurements were made with a Beckman model H-2 line-operated pH meter.

The results reported in this investigation were taken from studies covering a period of at least 1 year and thus are typical data indicating the behavior of milk samples in general. The milk was obtained from the University Dairy, Department of Dairy Husbandry, and was composited herd milk.

The heat-treated skimmilk samples were prepared in the following manner. Temperatures below 100° C. were maintained by means of a constant temperature water bath. A steam water bath was used for the samples heat-treated at 100° C. and an autoclave for temperatures above 100° C. A period of 20 to 25 minutes was required to bring the skimmilk to the desired temperature; it was then held for the respective time period and cooled with running water to room temperature within 5 to 8 minutes after removal from the autoclave or water bath.

The stability number measurements were conducted in accordance with a modification of the test of Storrs *(13)*, in which increments of 1/10 ml. of 0.1 *N* HCl were added to 10 ml. of the skimmilk sample in test tubes, and the tubes

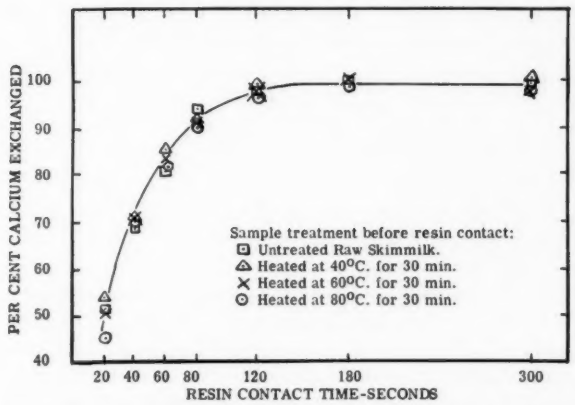

FIG. 1. The exchange of calcium from raw and heat-treated skimmilk. Thirty ml. of Amberlite IRC-50-K-H buffered at pH 6.8 were used.

were placed in a boiling water bath for 10 minutes. The test tube containing the smallest amount of added acid and which showed coagulation of the milk was selected as the indicator of protein stability. The number of tenths of milliliters of acid in the test tube was considered as the stability number for that sample.

RESULTS AND DISCUSSION

In preliminary experiments by the RCTM a decrease in calcium available for exchange to an ion-exchange resin was noted for heat-treated skimmilk samples. Thus, the following experiments were designed to study the effect of heat on the availability of calcium in samples of skimmilk subjected to temperatures of 40°, 60°, 80°, 100°, 110°, and 120° C. for various time periods. In Figure 1 data are presented showing the exchangeability of calcium in raw skimmilk and skimmilk heated to temperatures of 40°, 60°, and 80° C. for 30-minute periods. Very little decrease was noted in the exchange of calcium from these samples. Also, there was no marked change in the stability number, as shown in Table 1. Thus, it may be concluded that pasteurization temperatures do not cause a

TABLE 1

Temperature, heating time, pH, and stability numbers of skimmilk samples studied by the RCTM

Temperature	Heating time	pH	Stability No.
(° C.)	*(min.)*		
Skimmilk	—	6.77	7
40	30	6.81	7
60	30	6.82	7
80	30	6.80	7+
100	30	6.62	7+
110	30	6.60	9+
120	30	6.40	9+
120	45	6.39	8+
120	60	6.23	7

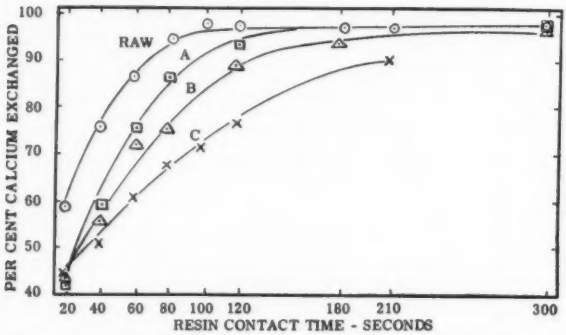

FIG. 2. The exchange of calcium from raw and heat-treated skimmilk. Sample treatment before resin contact: (A) Heated at 100° C. for 30 minutes, (B) heated at 110° C. for 30 minutes, (C) heated at 120° C. for 30 minutes. Thirty ml. of Amberlite IRC-50-K-H buffered at pH 6.8 were used.

decrease in the amount of calcium ions available to an ion-exchange resin. Further RCTM studies were made on skimmilk heated to temperatures of 100°, 110°, and 120° C. for periods of 30 minutes. As shown in Figure 2, the calcium available for exchange significantly decreased as the temperature of heating increased from 100° to 120° C. for holding times of 30 minutes. A comparison can be made for a particular contact time, for example, 60 seconds. However, as the resin-milk contact times increased to approximately 300 seconds, the amount of exchangeable calcium in heated skimmilk approached the values obtained for raw skimmilk. In the foregoing RCTM studies a 30-minute heating period was arbitrarily chosen, since this period of heating occurs in the process of manufacturing some milk products. A single lot of skimmilk was used for all equilibrations shown in Figures 2 and 3.

The following experiment was conducted in which longer periods of heating were employed, 45 and 60 minutes at 120° C. These results are presented in

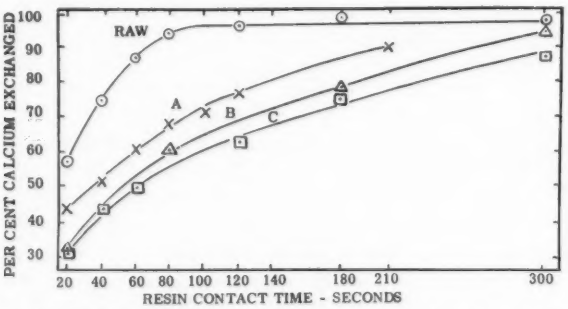

FIG. 3. The exchange of calcium from raw and heat-treated skimmilk. Sample treatment before resin contact: (A) Heated at 120° C. for 30 minutes, (B) heated at 120° C. for 45 minutes, (C) heated at 120° C. for 60 minutes. Thirty ml. of Amberlite IRC-50-K-H buffered at pH 6.8 were used.

Figure 3. The amount of calcium available for exchange from heat-treated skimmilk decreased as the heating time increased from 30 to 60 minutes at 120° C. It was also noted (Table 1) that the stability number of the skimmilk decreased and the product was noticeably brown after 60 minutes of heating at 120° C. An increase in the stability number was observed for skimmilk heat-treated at 110° and 120° C. for 30 minutes. However, upon holding the skimmilk at 120° C. for periods longer than 30 minutes, the stability number decreased as the duration of heating increased. This effect is probably due to the degradation of lactose and the production of lactic acid. Data are presented in Table 1 on temperature, duration of heating, pH, and stability numbers of all skimmilk samples studied in this investigation by the RCTM. A definite decrease was noted in the pH of all samples heated at temperatures above 80° C. for 30 minutes.

The effect of heat on the rate of removal of calcium from skimmilk is shown in Figure 4. Two temperatures of heating commonly used in commercial practices were chosen, 62° and 120° C. for 30 minutes. The graph was obtained by plotting the logarithm of the concentration of calcium remaining in the skim-

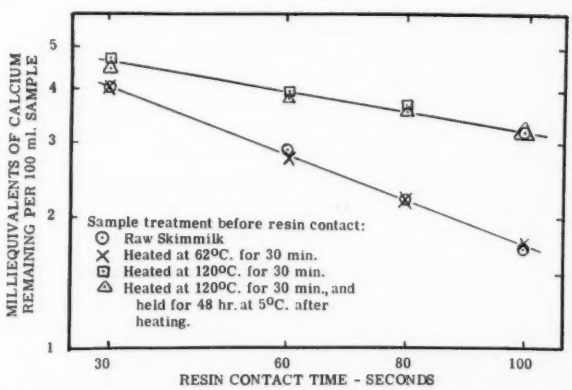

FIG. 4. The effect of heat on the rate of removal of calcium from skimmilk. Fifteen ml. of Amberlite IRC-50-K-H buffered at pH 6.8 were used.

milk sample after equilibration with the resin versus the contact time in seconds. Fifteen ml. of wet Amberlite IRC-50-K-H resin regenerated in the potassium form and buffered at pH 6.8 were used. In these experiments the ratio of milliequivalents of exchange capacity of resin to milliequivalents of total calcium in a 25-ml. sample of skimmilk is large, from 40:1 to 80:1, depending on the quantity of resin chosen. Also, experiments were conducted with different amounts of ion-exchange resin (10 to 40 ml. of damp resin) on the exchange of calcium from quaternary true solutions containing the cations common to milk and at similar concentrations. In all cases it was found that the removal of calcium was essentially complete in 10 to 20 seconds of contact time. This shows that the resin itself is not a rate-determining factor and that the rate of removal

of calcium from skimmilk is not affected by small changes in the amount of resin used. Resin contact times of 30 to 100 seconds were chosen in order that the most significant differences in rates of removal would be obtained. The exchange rate of removal for calcium was decreased by more than 50% for skimmilk heated at 120° C. for 30 minutes as compared to raw and pasteurized skimmilk. The exchange rate of removal of calcium was not appreciably altered by allowing the heated skimmilk to stand at 5° C. for 48 hours prior to resin contact-time studies. These data indicate that subjecting skimmilk to a temperature of 120° C. for a duration of 30 minutes results in a permanent shift in the equilibria of calcium, which does not change on aging.

Another experiment was conducted to determine the effect of heat on the rate of calcium removal in which 30 ml. of wet Amberlite IRC-50-K-H were used. Again, the skimmilk was heated to 120° C. for 30 minutes. Each point in Figure 5 represents the average value of two independent RCTM determinations on

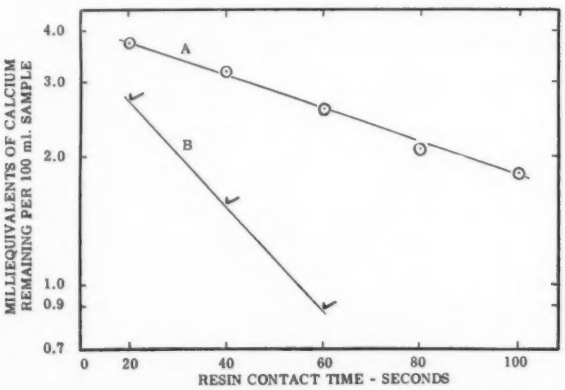

FIG. 5. The effect of heat on the specific rate constant for removal of calcium from skimmilk. Sample treatment before resin contact: (A) Heated at 120° C. for 30 minutes, (B) raw skimmilk. Thirty ml. of Amberlite IRC-50-K-H buffered at pH 6.8 were used.

separate samples of the same lot of skimmilk. The plot results in a straight-line relationship characteristic of a first-order reaction when the logarithm of the calcium remaining in the contacted sample is plotted versus the contact time in seconds. The specific reaction-rate constant or the velocity constant for the exchange of calcium from skimmilk can be calculated from the equation $k = \frac{2.303}{t_2 - t_1} \log \frac{C_1}{C_2}$; where C_1 and C_2 are the concentrations remaining in the skimmilk equilibrated with the resin for times t_1 and t_2, and k is the specific velocity content. The value of k for raw skimmilk was found to be 0.0118 second^{-1} and for heat-treated skimmilk 0.0039 second^{-1} (30 ml. of resin used).

It appears that the rate phenomenon involved for the exchange of calcium from raw or heated skimmilk is an over-all single first-order decomposition reaction within the time interval investigated. The decomposition reaction for

calcium phospho-caseinate and citrate complexes, including both dissolved and colloidal phases, is of such a nature that it behaves as a simple system with a single rate constant.

The inorganic balance in milk and the stability of the milk proteins can be significantly altered by heating. These changes may be due to dephosphorylation of casein, lower bound water content, increases in the acid precipitable serum proteins, and/or shifts in the inorganic equilibria. Any combination of these physico-chemical factors may influence the exchangeability of calcium from the milk to the resin. It has been definitely shown that heating skimmilk at 100° C. or above for 30 minutes decreases the availability of calcium. Also, as the temperature and time of heating skimmilk increase above 120° C. for 30 minutes, a decrease occurs in the stability number, as well as in the exchangeable calcium.

SUMMARY

The ion exchangeable calcium in raw and heated skimmilk was directly determined by the resin contact time method in order to study the effect of heat on the equilibria of the calcium.

Heating skimmilk at 40°, 60°, and 80° C. for 30 minutes had no discernible effect on the exchangeability of calcium. However, the exchangeable calcium was significantly decreased as the temperature of heating increased from 100° to 120° C. for holding times of 30 minutes. When longer periods of heating at 120° C. were employed (45 to 60 minutes), a further decrease was noted in the amount of calcium exchanged. It was found that the shift in the equilibria of calcium due to heat was permanent and did not revert on aging the milk at 5° C. for 48 hours.

The exchange rate of removal of calcium was decreased by more than 50% for skimmilk heated at 120° C. for 30 minutes as compared to raw and pasteurized skimmilk. The specific reaction rate constant for the exchange of calcium from raw skimmilk contacted with 30 ml. of IRC-50-K-H buffered at pH 6.8 was calculated to be 0.0118 second^{-1}, and 0.0039 second^{-1} for skimmilk heated at 120° C. for 30 minutes. It appears that the rate phenomenon involved in the decomposition reaction for calcium phospho-caseinate and citrate complexes in skimmilk, including both dissolved and colloidal phases, is of such a nature that it behaves as a simple system with a single rate constant.

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A CHROMATOGRAPHIC STUDY OF CHEDDAR CHEESE RIPENING[1]

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The amino acids and amines formed during cheese ripening can be used as a basis of measurement of the progress of the protein breakdown. A one-dimensional buffered filter paper chromatographic method of analysis was used to study protein changes during ripening of 30 samples of Cheddar cheese made from both raw and pasteurized milk. Eighteen amino acids were definitely identified, and the presence of others was suspected. *Editor.*

The ripening of Cheddar cheese is a complex process involving fermentation of lactose, degradation of proteins, hydrolysis of fats, production of volatile fatty acids, and other changes. This study is chiefly concerned with the decomposition of proteins, which may be progressively broken down to proteoses, peptones, polypeptides, amino acids, amines, and ammonia. To study the rate and extent of protein decomposition which is evidenced by changes in the body, texture, and flavor of the cheese, a method must be adopted to measure the concentration of the breakdown products. Since many of the compounds released in maturing Cheddar cheese are very complex and since the number of compounds is numerous, it is necessary to choose a group of the simpler compounds that can be more easily identified and measured. The amino acids and amines comprise such a group.

Several methods have been used in the past to determine these compounds in cheese. In 1903, Van Slyke and Hart *(9)*, in an effort to relate CO_2 production to proteolysis in the ripening of Cheddar cheese, found six amino acids and amines by tedious chemical analysis. In 1937, Tuckey *et al. (8)* identified four amino acids by means of an X-ray diffraction analysis. Later, Harper and Swanson *(3)* and Reihard and Garey *(7)* showed the presence of a number of amino acids in cheese by microbiological assay.

The foregoing methods of analysis are not well suited to the examination of a large number of cheeses since they are tedious and time-consuming. At the time these methods were used, the investigators identified only a few of the free amino acids that are now known to be liberated in Cheddar cheese. A notable advance was made in identifying these compounds in cheese when Kosikowski *(4)*, using paper partition chromatography, applied with minor modifications the two-dimensional method of Consden *et al. (1)* to the study of Cheddar cheese. Later, in

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[1] The data in this paper are from a thesis presented by the senior author in partial fulfillment of the requirements of the M.S.A. degree, University of Toronto.

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1953, Lindqvist *et al.* of Stockholm *(5)* reported using a one-dimensional buffered filter paper chromatographic method of analysis for cheese based on the method of McFarren *(6)* with minor modifications. This method is essentially the one used in this work to identify the amino acids and some amines. Hereafter, these two closely related groups of compounds will be referred to collectively as amino acids.

METHODS

Thirty samples of Canadian Cheddar cheese made from both raw and pasteurized milk were analyzed for amino acids. They were obtained from the Ontario Agricultural College Dairy Department and from two western Ontario cheese storages and ranged in age from 2 weeks to 2½ years. The average curing temperature was about 7° C. The cheeses were graded by federal cheese graders and, except for three samples, were all first-grade by Canadian standards.

Preparation of cheese sample. Five g. of cheese sample were placed in a Waring blendor and comminuted for 2 minutes with 75 ml. of distilled water. After the pH of this mixture was adjusted to 6.2, the contents were washed into a 100-ml. graduate, diluted up to the mark with water, cooled to 13° C., and filtered through a Whatman No. 12 fluted filter paper to remove the fat and undissolved casein. Fifty ml. of the filtrate were added to 250 ml. of 95% ethanol, which caused any remaining proteins and peptides to be precipitated. On standing overnight the precipitate settled out, and the alcoholic extract was decanted off and filtered through a Whatman No. 12 fluted filter paper. Two hundred ml. of the filtrate were then evaporated to dryness with warm air. The resultant residue, which represented 1.67 g. of cheese, was then dissolved in 5 ml. of distilled water. This solution was used for spotting the Whatman No. 1 filter papers.

Preparation of amino acid standards and buffered solvents. A supply of amino acids comparable to those suspected of being in Cheddar cheese was obtained from Nutritional Biochemicals and from it aqueous standards were made up, to aid in the identification of the amino acids as they appeared on the chromatograms. All the solutions were adjusted to pH 6.2 and most of them were of a 4 m*M* concentration.

The four aqueous buffers required had pH values of 6.2, 8.4, 9.0, and 12.0 and were prepared in a similar manner to those of McFarren *(6)* except that a 0.1% addition of a commercial alkyl aryl sulfonate was made as suggested by Lindqvist *et al. (5)*. These buffers, when used to buffer the four solvents chosen, gave the following five combinations: phenol at pH 12.0; collidine, pH 9.0; *m*-cresol, pH 9.0; *m*-cresol, pH 8.4; and *o*-cresol, pH 6.2. The same buffers were used to buffer the filter papers.

Spotting the filter paper. The amount of solution spotted on the buffered Whatman No. 1 filter paper (18 × 22.5 in.) varied from 0.0025 to 0.015 ml., depending on the age of the cheese. The spots were applied 1 in. apart on a lightly drawn pencil line 2.5 in. from the narrow end of the filter paper.

Chromatographic chambers and drying equipment. The five chromatographic chambers necessary were made of ⅝-in. plywood and measured 12 × 24 and

23 in. high, outside dimensions. Each chamber was air-tight and had a glass front. A stainless steel trough capable of holding enough solvent for two chromatograms (75 ml.) was supported near the top of each chamber. The chambers were placed in an insulated room thermostatically controlled at 25° C. ± 1°.

A drying cabinet was used to dry the filter papers by drawing air at room temperature over them. When dry, the papers were sprayed with 0.2% ninhydrin in 95% ethanol to color the amino acid spots. The reaction between the amino acids and the ninhydrin was brought to completion by hanging the papers in a 65° C. oven for 15 minutes.

Qualitative and quantitative determinations. The amino acids separated were qualitatively determined by ascertaining the Rf values and by comparing the spots with standards spotted on the same filter paper. Quantitative estimations involved the use of an "Eel" photoelectric cell scanner that was manufactured by Evans Electroselenium Ltd. of Sussex, England. A scanner value was obtained for each amino acid, which was then converted to milliliters of standard acid solution by comparing the values to previously prepared standard curves. A standard curve was made for each compound except asparagine, proline, and glycine by spotting increasing concentrations in replicate and developing the chromatograms with the particular buffered solvent that proved most effective in separating the amino acid.

Amino acids separated by buffered solvents. The combinations of solvents and buffers together with the amino acids best separated by them were as follows:

phenol pH 12.0—aspartic acid, glutamic acid, glycine, asparagine, serine, alanine, threonine, and tyrosine
m-cresol pH 9.0—histidine and lysine
m-cresol pH 8.4—arginine, leucine, isoleucine, and proline
o-cresol pH 6.2—methionine
collidine pH 9.0—tyramine, valine, and phenylalanine

RESULTS AND DISCUSSION

Thirty samples of Canadian Cheddar cheese were examined for free amino acids. In order to obtain as complete a picture as possible, cheeses were chosen that had been cured for as long as 2½ years.

Eighteen amino acids were identified with certainty, and the presence of others was suspected. Tables 1 and 2 indicate the concentrations of the amino acids that were detected in 18 of the cheeses analyzed. Not all compounds identified were present in all the cheeses examined. Where the concentrations of amino acids were too small for reliable scanner readings to be obtained, or where the separations were inadequate, their presence is indicated by plus signs. Negative signs indicate the acids that could not be detected.

The amino acids that were detected in all the cheese samples were: glutamic acid, aspartic acid, leucine, methionine, lysine, valine, alanine, phenylalanine, threonine, and tyrosine. Serine was not found in two of the younger cheeses and was surprisingly absent in a 6-month-old raw milk cheese. The basic amino acids,

TABLE 1

Free amino acids in raw milk Canadian Cheddar cheese cured at an average temperature of 7° C.

Age in days:	17	60	90	120	190	235	330	555	900
Flavor score:	40	39	39	39	39	40	39	37	40
Cheese No.	6	14	16	15	12	5	7	31	32
Amino acid	*(Mg/g cheese)*								
Alanine	0.2	0.67	0.52	1.2	1.1	1.1	4.2	3.2	4.2
Arginine	−	1.2	1.6	2.7	+	−	1.7	+	++
Asparagine	−	+	+	+	−	−	−	−	−
Aspartic acid	0.12	0.12	0.08	0.24	1.3	1.6	5.9	+++	+++
Glutamic acid	2.0	1.5	2.1	3.6	4.5	4.6	10.7	18.4	19.4
Glycine	−	+	+	+	+	+	+	++	+
Histidine	−	−	−	−	−	+	1.2	2.4	1.6
Leucine	2.0	1.9	3.4	4.8	3.7	2.6	8.8	10.2	10.3
Lysine	1.0	+	+	1.1	3.4	2.1	0.91	6.2	10.1
Methionine	0.2	0.48	0.28	0.90	0.63	0.90	2.2	3.9	5.5
Phenylalanine	1.1	1.1	1.5	1.6	3.6	2.5	4.9	3.2	3.2
Proline	−	−	−	−	+	+	++	−	++
Serine	+	+	0.12	0.14	0.45	0.24	2.0	2.2	2.8
Threonine	0.14	+	0.24	0.49	0.48	0.70	5.3	2.8	1.8
Tyramine	−	−	−	−	0.85	+	3.7	1.7	2.2
Tyrosine	0.2	0.25	0.30	0.34	0.54	1.1	5.1	1.0	1.2
Valine	0.8	0.74	0.90	2.1	2.1	1.9	4.8	8.0	8.0

arginine and lysine, which had been reported previously as a group *(2, 4)*, were individually separated. Arginine was first detected in raw milk cheese at 60 days of age and in pasteurized milk cheese at 130 days of age. Lysine, however, was present at a very early age. Therefore, it would appear that the early appearance of this basic amino acid group in previous work was due solely to the presence

TABLE 2

Free amino acids in pasteurized milk Canadian Cheddar cheese cured at an average temperature of 7° C.

Age in days:	16	35	130	200	300	330	400	560	835
Flavor score:	39	39	40	39	41	41	40	38	37
Cheese No.	8	13	28	28	24	21	22	30	29
Amino acid	*(Mg/g cheese)*								
Alanine	0.5	0.57	0.68	1.0	1.6	1.6	2.6	2.5	5.0
Arginine	−	−	0.48	2.4	−	−	−	++	++
Asparagine	−	−	+	++	++	++	++	+++	+++
Aspartic acid	0.21	+	0.13	0.15	2.4	0.55	1.2	++	++
Glutamic acid	2.1	1.1	3.9	4.4	6.2	8.5	12.0	10.5	10.8
Glycine	−	+	+	++	++	++	++	+++	+++
Histidine	−	−	−	+	−	0.66	−	1.4	1.8
Leucine	1.0	2.2	1.1	3.3	5.7	5.8	6.6	9.4	9.8
Lysine	0.21	1.0	0.66	1.5	3.2	2.4	10.6	3.1	4.1
Methionine	+	0.22	0.20	0.63	2.4	2.2	3.4	2.5	3.9
Phenylalanine	0.54	0.90	2.4	2.4	3.4	2.4	3.3	2.5	3.0
Proline	−	−	−	+	+	+	+	++	++
Serine	0.18	−	0.40	0.52	0.82	0.55	1.7	3.4	5.5
Threonine	0.70	+	0.32	0.48	0.85	0.63	1.3	1.3	2.3
Tyramine	−	−	−	−	+	+	+	−	0.80
Tyrosine	0.48	0.39	0.34	0.38	1.3	1.1	1.6	1.1	1.1
Valine	0.60	1.3	1.7	3.7	3.4	3.0	4.4	6.8	10.8

of lysine. Histidine was detected in raw and pasteurized milk cheese at about 170 days of age.

Leucine and isoleucine were easily separated as a group but were not completely separated from each other. Consequently, their total is reported as leucine. Glycine was found in all cheeses except those under the age of 5 weeks. Proline appeared at about 180 days in both raw and pasteurized milk cheese. It is possible that this amino acid was present at an earlier date but, owing to its light yellow color, was passed unnoticed. Tyramine was found in raw milk cheese at 6 months of age and also in three pasteurized milk cheese samples, the youngest of which was 8 months old. Asparagine, in addition to being found in all but two very young pasteurized milk cheeses, was also found in four raw milk cheese samples ranging in age from 2 to 6 months. Previously it had been reported only in pasteurized milk cheese.

On several *o*-cresol and collidine chromatograms the presence of alpha and gamma amino butyric acids was suspected but was not conclusively proven. Spots were also observed on a few chromatograms that could not be attributed to any specific amino acid.

Quantitatively, phenylalanine, serine, alanine, aspartic acid, and threonine all increased in concentration until the cheeses were about 1 year of age and then appeared to remain about the same, whereas valine, lysine, leucine, methionine, and glutamic acid continued to increase with the age of the cheese. The concentrations of arginine and histidine when detected did not appear to vary a great deal with the age of the cheese. Tyramine and tyrosine did not seem to increase in the cheese after the age of 6 months. The limitations of the analytical method in not allowing tyrosine to become more fully soluble prevented this amino acid from being accurately estimated. Therefore, even if large concentrations were present in the cheese, only the amount that would saturate a water solution would be found in the extract.

The results obtained from pasteurized milk cheese indicate that the trends of liberation of many of these amino acids were similar to those in raw milk cheese. The concentrations of alanine, aspartic acid, methionine, phenylalanine, and threonine all increased until the age of 8 months, at which time they leveled off. Arginine and histidine gave results similar to those in Table 1. Lysine and glutamic acid continued to increase for 12 months, and leucine, valine, and serine continued a general increase. Glycine, proline, and asparagine also appeared to increase with age.

It is not possible to make more than general comparisons between the data in Tables 1 and 2 since most of the cheeses were made from different milk, in different factories, and under widely divergent conditions. The curing and ages of the cheese also were different. However, examination of the tables shows that some amino acids reach greater concentrations in raw milk cheese than in pasteurized milk cheese and vice versa. Aspartic acid, leucine, and lysine were generally present in larger concentrations in raw milk cheese. This was true also of glutamic acid in the older samples. Pasteurized milk cheese had more serine and asparagine present.

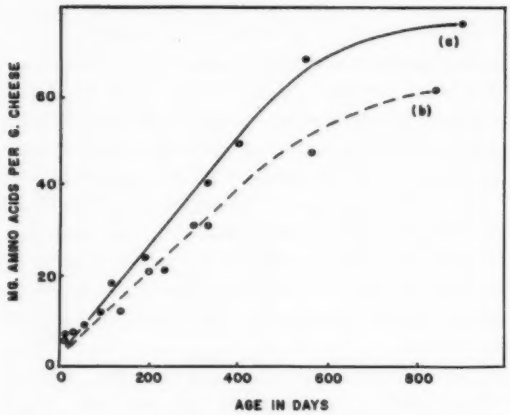

FIG. 1. Comparison of the total free amino acid content of raw (a) and pasteurized milk cheese (b) as observed by paper chromatography.

Figure 1 shows a comparison between the total amounts of free amino acids measured in raw and pasteurized milk cheese. When two or more cheeses of similar age were analyzed, average values were plotted.

Table 3 shows two pasteurized milk cheeses which were analyzed for free amino acids at the ages of 4, 6½, and 8½ months. These cheeses were similar except that a culture of *Lactobacillus casei*, #350 O.A.C., was added to the milk

TABLE 3

Free amino acids in two pasteurized milk Canadian Cheddar cheeses at three different ages and cured at 10° C.

	Cheese 25			Cheese 28		
	Months			Months		
Amino acid	4	6½	8½	4	6½	8½
	(Mg/g cheese)					
Alanine	0.54	0.58	0.58	0.68	1.0	1.1
Arginine	−	1.6	2.5	0.48	2.4	3.5
Asparagine	+	++	++	+	++	++
Aspartic acid	0.12	0.15	0.15	0.13	0.15	0.21
Glutamic acid	2.8	3.6	5.3	3.9	4.4	6.6
Glycine	+	++	++	+	++	++
Histidine	−	−	−	−	−	−
Leucine	1.1	3.2	4.4	1.1	3.3	5.8
Lysine	0.92	0.85	++	0.66	1.5	++
Methionine	0.40	0.45	0.55	0.20	0.63	1.1
Phenylalanine	2.3	1.9	1.8	2.4	2.4	3.1
Proline	−	+	+	−	+	++
Serine	0.24	0.38	0.42	0.40	0.52	0.58
Threonine	0.32	0.33	0.36	0.32	0.48	0.42
Tyramine	−	−	−	−	−	−
Tyrosine	0.25	0.30	0.39	0.34	0.38	0.60
Valine	1.6	3.0	1.7	1.7	3.7	4.0

from which No. 28 was made in an effort to stimulate amino acid liberation. At the age of 4 months there did not seem to be too great a difference in the free amino acid content of the two cheeses except that No. 28 was substantially richer in glutamic acid, serine, and tyrosine. At 6½ and 8½ months all the amino acids had increased in concentration with the possible exception of aspartic acid and phenylalanine, which appeared to remain the same, whereas serine, valine, and threonine increased only slightly. At 8½ months it was quite evident that amino acids were being liberated at a greater rate in cheese No. 28 than in No. 25. Tyramine and histidine were not detected in these cheeses.

SUMMARY

The one-dimensional buffered filter paper chromatographic method of analysis with five differently buffered solvents has proven satisfactory for the determination of free amino acids in Cheddar cheese. Eighteen amino acids were identified with certainty, and the presence of others was suspected. Quantitative estimations were obtained for most of the separated amino acids by using a photoelectric cell scanner in conjunction with standard curves. In general, amino acid concentrations were found to increase with age. Also, the concentrations were generally greater in raw milk cheese than in pasteurized milk cheese of a comparable age.

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RADIATION PRESERVATION OF MILK AND MILK PRODUCTS. IV. RADIATION-INDUCED BROWNING AND SOME RELATED CHEMICAL CHANGES IN MILK[1]

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The browning of milk has long been a problem in the manufacture of highly heated dairy products. In the application of ionizing radiation milk browned rapidly when heated to 100° C. The study reported has to do with the nature of this change. Radiation-induced browning was prevented when carbonyls were eliminated from the irradiation system. *Editor.*

Nonenzymatic browning of milk is known to occur when milk is stored for prolonged periods at elevated temperatures and when it is heated for longer than 60 minutes at 100° C. *(13)*; browning has also been observed during the production of evaporated milk and of spray dried milk *(6, 7, 19)*.

The browning reaction, both generally and with particular reference to milk, has been studied by many investigators, and a comprehensive review of the research on browning in milk has recently been published by Patton *(10)*. Investigations into the application of ionizing radiations to milk revealed that a sensitivity to browning was induced, causing milk to brown rapidly when heated to 100° C. The present paper is concerned with studies of the nature of this radiation-induced change.

MATERIALS AND METHODS

The equipment and the radiation technique used in this investigation were the same as those described in a previous report *(20)*.

Reflectance measurements of milk and milk products were made with a rapid scanning spectrophotometer *(11)*, which gave spectrophotometric curves (indicated by an electronic cathode ray tube) in the region of 400 to 700 mμ.

Transmittance measurements were made with a Beckman Model DU spectrophotometer, in which 1-cm. Corex cells were used for measurements in the visible region of the spectrum and 1-cm. silica cells for transmittance in the ultraviolet region. The method of Choi *et al. (1)* was used for measurement of browning by transmittance and was found to produce consistent results. The suggestion of Patton *(9)* to increase the amount of trypsin was followed by using 1.5 ml. of a 13% dispersion of trypsin.

Unirradiated control samples and irradiated samples were pipetted in 25-ml. amounts into specimen vials of 35-ml. capacity and immersed for exactly 20

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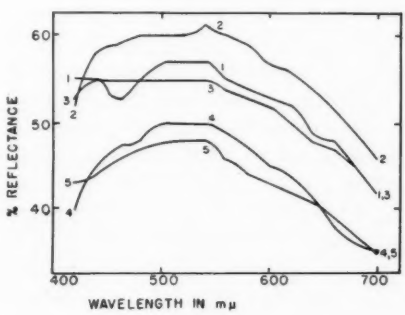
minutes in a boiling water bath. The vials were then cooled rapidly in ice water. Trypsin digestion and precipitation with trichloroacetic acid were next carried out as suggested by Choi *et al. (1)*. Approximately 0.5 g. of Hyflo-Supercel was used to aid in the filtration of the serum, and the first few milliliters of the filtrate were returned to the filter to insure clear filtration.

Pasteurized nonfat milk and pasteurized homogenized whole milk obtained from a commercial dairy were used throughout this work unless otherwise specified.

RESULTS AND DISCUSSION

Preliminary observations by the authors showed that radiation-induced bleaching occurred not only in whole milk but also in nonfat milk, whereas it did not occur in solvent-extracted milk residues[2] or lactose-casein model systems. All nonfat systems containing lactose and casein, however, exhibited within the first week after irradiation a brown coloration (browning) on storage at 40° F. Neither irradiated whole milk nor irradiated homogenized whole milk browned on storage at 40° F. Further investigation, however, revealed that the protective action of lipids existed only at low temperatures, and when irradiated milk was heated to 100° C. browning seemed to start within minutes. The intensity of browning progressed with the time of heating and seemed to be the same for skimmilk, irradiated whole milk, and homogenized milk. When the heating time remained constant, the intensity of browning progressed with increasing dose. At low dose levels, however, the amount of bleaching seemed to cancel out the production of browning precursors.



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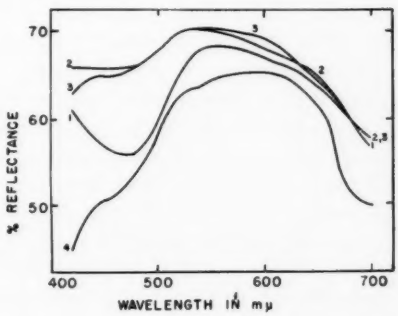

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FIG. 1. Reflectance curves of nonfat milk stored at 40° F. after irradiation with 2×10^6 rep. Curve 1, unirradiated control; curve 2, irradiated (no storage); curve 3, irradiated and stored 5 days; curve 4, irradiated and stored 9 days; curve 5, irradiated and stored 14 days.

FIG. 2. Reflectance curves of homogenized whole milk stored at 40° F. after irradiation with 2×10^6 rep. Curve 1, unirradiated control; curve 2, irradiated (no storage); curve 3, irradiated and stored 14 days; curve 4, irradiated and heated for 5 minutes at 100° C.

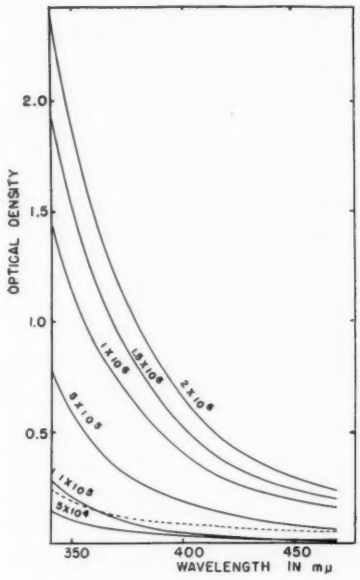
[2] Milk residues that had been freed of lipoids by extraction with ethanol, petroleum ether, and diethyl ether *(21)*.

Effect of high-energy cathode rays on color of nonfat milk. Samples of nonfat milk were irradiated by high-energy cathode rays at a dose level of 2×10^6 rep. The samples were then stored at 40° F. for 14 days, and within that period reflectance measurements were made in the spectral region of 400 to 700 mμ. Curve 1 in Figure 1 shows that the unirradiated nonfat milk had a maximum reflectance in the region of 500 to 540 mμ and a minimum reflectance at 460 mμ. Similar locations of maximum and minimum were found with whole milk and homogenized milk. The bleaching effect caused by irradiation (curve 2) was shown as an over-all increase in reflectance above 420 mμ. Browning was observed (curves 3, 4, and 5) on storage at 40° F., and the intensity of the browning increased with time of storage. Reflectance measurements showed that the original coloration was reached within 5 days (curve 3), although the reflectance minimum at 460 mμ remained irreversibly destroyed. Progressive darkening was noted (curves 3, 4, and 5) on further storage.

Effect of high-energy radiations on color of homogenized milk. Irradiation of homogenized milk with 2×10^6 rep caused destruction of the reflectance minimum in the region of 460 mμ (Figure 2). This finding is similar to the observations made on skimmilk (Figure 1). On storage at 40° F., no appreciable browning was noted (Figure 2, curves 2 and 3) such as indicated by the similarity of the corresponding reflectance curves in Figure 1. When a sample of irradiated homogenized milk was heated at 100° C. for 5 minutes, appreciable browning was observed (Figure 2, curve 4). When doses lower than 2×10^6 rep were applied, the reflectance measurements of radiation-induced browning met with some difficulties because bleaching and browning effects showed some tendency to cancel each other.

This phenomenon was resolved by measuring the intensity of browning according to the tryptic digestion method of Choi *et al. (1)*. Unirradiated control samples were used as solvent blanks. The optical densities of homogenized milk irradiated at various dose levels and then heated for twenty minutes at 100 °C. and digested are shown in Figure 3 as Δ E (the optical density of the sample as measured against the density of the unirradiated control as the solvent blank). The spectrum of the unirradiated control sample (shown in broken line in Figure 3) is expressed as E (the optical density of the control referred to water as the solvent blank). No absorption maximum was found in the visible spectrum. The curves of the densities at lower dose levels (5×10^4 and 1×10^5 rep) crossed each other at 430 mμ, which indicates that the bleaching effect of the dose level of 5×10^4 rep was greater than the radiation-induced browning. To evaluate relative intensities of browning, choice of a lower wave length was indicated. The optical density values at 360 mμ were found to be most nearly proportional to dose and, therefore, measurements of relative browning intensities were made at this wave length.

When 25-ml. portions of homogenized milk irradiated with 2×10^6 rep were heated for various periods of time, increasing optical densities were found at 360 mμ (Figure 4). Standardization to 20 minutes' heating time was chosen, in order to read the optical densities within the flatter portion of the curve. The



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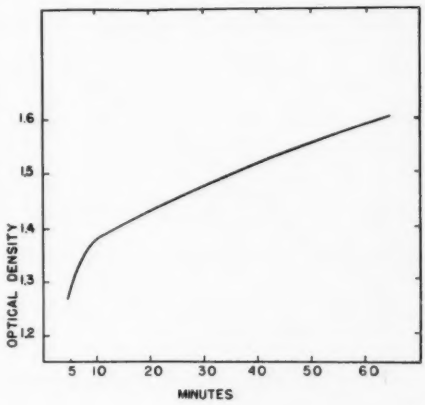

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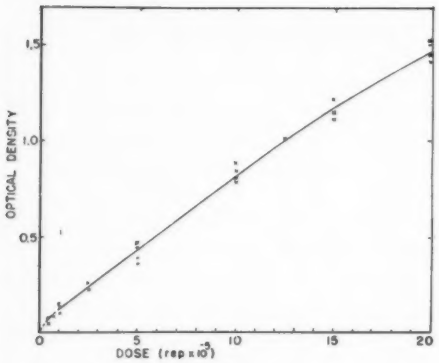
FIG. 3. Browning reaction of irradiated homogenized milk. Absorption spectrum of serum with variable dose.

FIG. 4. Browning reaction of homogenized milk irradiated with 2×10^6 rep and heated at 100° C. for various lengths of time.

Δ E values of several determinations on homogenized milk obtained at 360 mμ for dose levels between 5×10^4 and 2×10^6 rep plotted against optical density are shown in Figure 5. The curve tends to flatten at higher dose levels. This behavior is taken as an indication that the indirect action of free radicals is responsible for the production of browning precursors. These components would, at higher dose levels, be produced in sufficiently high concentration to compete with the unreacted molecules of the solutes as acceptors for the free radicals. This secondary reaction would be expressed as a proportionally lower browning intensity at higher dose levels (Figure 5).

Skimmilk, whole milk, and homogenized milk irradiated with equal doses developed the same intensity of brown coloration after heating and after tryptic digestion.

Proctor and O'Meara *(13)* have shown that free radicals can be immobilized by irradiation of milk in the frozen state. A similar experiment was performed in this investigation, and the browning reaction was used as the indicator. When the browning reaction took place in homogenized milk after irradiation with 1×10^6 rep, the optical density at 360 mμ (Δ E) was found to be 0.792. The Δ E value of frozen milk (packed in dry ice) irradiated at the same dose level was reduced to 0.064 (8% retention of browning sensitivity). According to the values



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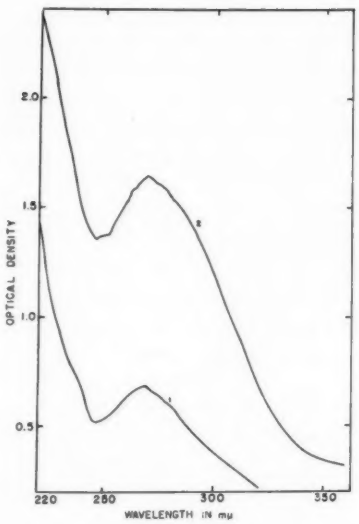

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FIG. 5. Browning reaction of irradiated homogenized milk. Relation of optical density at 360 mμ to dose.

FIG. 6. Optical densities of lactose solutions in phosphate buffer after irradiation with 2×10^6 rep. Curve 1, after irradiation; curve 2, after irradiation and heating at 100° C.

shown in Figure 5, this would correspond to the sensitivity induced by the application of approximately 5×10^4 rep.

Vacuum distillation of the off-flavor components *(20)* did not change the intensity of browning in the reconstituted milk residue.

Origin of radiation-induced browning and thiobarbituric acid (TBA) reactions. In an earlier publication *(20)* it was reported that some part of the thiobarbituric acid pigments absorbing at 535 mμ was found to originate with milk lipids. However, the larger amount of TBA pigmentation at 500 mμ and at 535 mμ was derived from chemical changes within the lactose-protein group. The similarity of origin of TBA reactants and browning precursors suggested the desirability of simultaneous study of both reactions. Accordingly, 3% casein (Hammersten grade) was dispersed in solutions containing 4% lactose (reagent grade) dissolved in a 0.1 *M* K_2HPO_4-NaH_2PO_4 buffer of pH 6.7. Bentonite (2.5%) was used as a dispersing agent. A mixed citrate-phosphate buffer more nearly represents the buffer system in milk. However, it was believed that citrates might be affected per se by ionizing radiations and thus might possibly contribute to some of the side reactions. Therefore, the phosphate buffer system alone was used. Irradiation of these dispersions with a dose of 2×10^6 rep was found to produce intense browning as well as TBA reacting substances.

Investigations were made to determine the relative role of each of these components with respect to each other, insofar as radiation-induced activity is

concerned. Solutions of lactose in phosphate buffer and dispersions of casein in phosphate buffer were prepared, to be combined after one or both parts had been irradiated. The resulting samples, therefore, contained equal concentrations of all components corresponding to milk, diluted approximately in half. The pH of these solutions had not changed after irradiation. Browning and TBA reactions were studied with the combined samples, the former reaction by Choi's method and the latter by the method described earlier *(20)*. The irradiated samples received a dose of 2×10^6 rep, and the following combinations of components were tested:

a) Irradiated casein and untreated lactose
b) Untreated casein and irradiated lactose
c) Irradiated casein and irradiated lactose
d) Casein and lactose irradiated together
e) Untreated casein and lactose were used as controls

The results of browning and TBA reactions are presented below in terms of Δ E values.

Sample	Browning Δ E at 360 mμ	TBA Δ E at 500 mμ
1. Lactose and irradiated casein	0.054	0.028
2. Casein and irradiated lactose	0.540	1.126
3. Computed densities of 1 and 2	0.594	1.154
4. Irradiated casein and irradiated lactose	0.578	1.174
5. Irradiated dispersion of casein in lactose	1.014	1.724

From these results it may be concluded that the radiation-induced production of precursors to browning and to TBA reactivity was confined to the time during irradiation and did not extend beyond the time when the components were combined (approximately 30 minutes after irradiation). At this time no further production of precursors seemed to take place, and the optical densities computed from samples 1 and 2 approximated the values found by the reaction of the combined irradiated components (sample 4). Therefore, there was no after-effect with respect to these reactions.

Irradiation of casein alone did not produce appreciable amounts of browning or of TBA precursors. The considerable increase in the intensity of reactions found when casein was irradiated in the presence of lactose (sample 5) would indicate that reactive intermediaries of these two substances caused the additional formation of precursors.

When casein was eliminated from sample 1, the irradiated lactose solution in phosphate buffer also browned on being heated for 20 minutes and gave a Δ E value of 0.620. This nonnitrogenous browning reaction was relatively less intense than the comparison with sample 2 would indicate. This may be ac-

counted for by the fact that Hammersten casein gave only incomplete digestion with trypsin, and not all the pigment was released. When casein was replaced with 2% methionine as one of the amino acids more susceptible to browning *(2)*, the Δ E value corresponding to sample 2 was found to be 1.140. Hence, amino groups do not seem essential to browning but enhance it.

Investigators of the browning reaction of milk found glucose to be the reactive part of the lactose molecule *(7, 10)*. Ionizing radiations caused sensitivity to browning in lactose as well as in glucose and galactose. When 0.1 *M* solutions of these sugars were irradiated with 2×10^6 rep and heated at 100° C. for 20 minutes after the addition of 0.1 *M* methionine, optical densities of 1.07 for lactose, 1.26 for galactose, and 0.93 for glucose were obtained. The browning density of a solution containing 0.1 *M* glucose and 0.1 *M* galactose, treated in a similar way, was found to be 1.95. The part of the sugar molecule that is sensitized to browning by ionizing radiations is not known at this time. Solutions of these sugars in water, both with phosphate buffer and with borate buffer, gave TBA reactions after irradiation, and they browned when heated for 20 minutes with casein dispersions.

The samples with radiation-induced browning of the Maillard type displayed intense fluorescence in ultraviolet light. The nonnitrogenous caramelization of irradiated lactose, on the other hand, showed no ultraviolet fluorescence. Further experiments now under way will determine whether both types of browning take place in irradiated milk, although the occurrence of simultaneous browning of Maillard and caramelization types is suspected.

Investigation of browning precursors. Neither milk nor buffered lactose solutions showed measurable changes of pH after irradiation with doses up to 2×10^6 rep. Titration against phenolphthalein as an indicator revealed that 7.5 μM of functional carboxyl groups per milliliter had been formed when buffered solutions of 4% lactose were irradiated with 2×10^6 rep. The nature of these carboxylic acids has not been determined at this time. Seaver and Kertesz *(17)* found uronic acids to be very sensitive to browning. Consequently, their formation during irradiation might be suspected. However, no increase in browning under these experimental conditions (on heating for 20 minutes) was observed when 1% glucuronic acid, as well as 1% galacturonic acid, was added to buffered casein-lactose solutions. It seems doubtful, therefore, that the carboxyl groups of carboxylic acids are involved in the radiation-induced sensitivity to browning.

When lactose solutions in phosphate buffer were irradiated with 2×10^6 rep, the spectrum in the ultraviolet region revealed the formation of substances with strong absorption at 268 mμ (Figure 6, curve 1). Heating for 20 minutes at 100° C. caused browning, and the spectrum showed further increase in optical density without shift of the maximum absorption.

A comparison of the spectra of hydroxymethylfurfural and of heated sugar solutions as studied by Singh *et al. (18)* and by Wolfrom *et al. (21)* reveals that the secondary maximum of furans at 230 mμ and the main absorption at 284-285 mμ, which are characteristic for the thermal breakdown of sugars, were

not produced in the radiation-induced browning. This finding was confirmed when irradiated lactose and whey from irradiated milk were extracted with diethyl ether.

No furan reaction was observed with resorcinol in HCl (Seliwanoff test). This furan test remained negative when extracts of browned irradiated samples were tested. These findings follow the pattern described by Proctor and Goldblith *(12)*, who reported the presence of hydroxymethyl furfural in prunes, raisins, prune juice, and dried brown apricots. They observed the destruction of this compound when these food products were irradiated with ionizing radiations.

The carbonyls formed by irradiation of buffered lactose solutions were not extractable with diethyl ether, benzene, or ethyl acetate. The irradiated aqueous solutions, however, formed crystalline hydrazones with 2,4-dinitrophenylhydrazine (0.5% solution of 2,4-DNPH in 2 *N* HCl). These hydrazones were extracted with ethyl acetate, and determinations were made according to the method of Henick *et al. (4)*. The results show that irradiation (2×10^6 rep) of a solution containing 111.2 μM of lactose per milliliter yielded 0.58 μM per milliliter of unsaturated carbonyls and 1.86 μM per milliliter of saturated functional carbonyls.

As it was considered that the precursors of the browning reaction were within the above group of carbonyl compounds, attempts were made to separate their semicarbazones or their bisulfite addition compounds, in order to gain proof of the browning reactivity of these components. Crystallization failed, but this failure may have been due to the extremely low concentration of these components.

An indirect proof of the role of carbonyls in the browning reaction, however, was obtained by elimination of all carbonyl compounds and by observation of the subsequent browning behavior of the carbonyl-free whey. Whey from irradiated (2×10^6 rep) milk was allowed to react with an excess of 2,4-DNPH (in 2 *N* HCl) by heating for 5 minutes at 100° C. The excess reactant, as well as the hydrazones, was repeatedly extracted with ethyl acetate. Controls were prepared by substituting 2 *N* HCl for the DNPH solution. After solvent extraction, all samples were brought to pH 6.7, and 5-ml. portions were added to 10 ml. of a solution containing 4% lactose and 2% methionine in 0.1 *M* phosphate buffer. This mixture was heated for 20 minutes at 100° C. The results obtained were as follows:

Sample	Δ E at 360 mμ
Sample 1. Whey from irradiated milk	0.420
Sample 2. Whey from irrad. milk treated with HCl and extracted	0.417
Sample 3. Whey from irrad. milk treated with 2,4-DNPH and extracted	0.013

Sample 1 (whey from irradiated milk) caused browning of the model system. Sample 2 (whey from irradiated milk treated with 2 *N* HCl—used as the control

for sample 3) also browned. However, sample 3 (whey from irradiated milk from which the carbonyls were separated as hydrazones) showed an insignificant browning reaction. This indirect evidence shows that irradiation-induced browning precursors are carbonyl compounds.

Reductones have been found in significant quantities in heat-induced browning reactions and are believed to be an important link in the reaction chain leading to brown pigments *(5)*. Therefore, it was deemed desirable to investigate the presence of reductones in irradiation-induced browning. It was observed that irradiated milk reduced 2,6-dichlorophenol indophenol. Lactose-casein systems, after irradiation, also reduced this dye. In fact, irradiated lactose solutions per se (in the absence of amino compounds) will also reduce this dye. Hence reductone-like compounds are produced by irradiation from the sugar fraction of the milk before browning is induced.

These reductones, when heated in the presence of amino acids, would be expected to form dicarbonyls by dehydration of the type described by Schonberg *et al. (16)* and to enter into Strecker degradation with the formation of carbon dioxide. However, irradiation of a lactose-methionine model system in ampoules and heating for 20 minutes at 100° C. produced browning, but when the system was tested in a micro gas-analysis apparatus, no traces of carbon dioxide were detected. The radiation-induced browning mechanism, at least in the first stages of browning, did not seem to proceed through a Strecker degradation.

The tendency to browning of irradiated lactose-containing systems at relatively low temperature suggested that the reaction mechanism might differ in some respects from that of the heat-induced browning. The precursors that form as a result of irradiation await isolation and identification.

Observations on the study reported would indicate that the browning precursors are polyhydroxycarbonyls. It may be that these compounds are formed by sugar dehydration in the presence of oxidative free radicals produced by the action of ionizing radiations on water *(3)*. Similar catalytic actions of O_3 and of H_2O_2 in browning systems have been observed by Rice *et al. (14)* and Schonberg and Moubasher *(15)*. The production of reductones by Amadori rearrangement and subsequent dehydration, as explained by Hodge and Rist *(6)*, might not be limited to N-substituted glycosides but could be induced by the high-energy input of ionizing radiations.

Certain similarities of the precursors for browning and thiobarbituric acid reactions were observed, although these precursors did not seem identical: *(a)* The production of both these groups increased with increasing irradiation doses. *(b)* They were found to be nonvolatile components, containing active carbonyl groups, and lactose contributed the greatest part to their formation. *(c)* TBA precursors were found to diminish on storage *(20)*, whereas the rate of browning (under experimental conditions) remained constant on storage so that the same ΔE was produced after 20 minutes.

The chemical composition of all these components is not known at this time. However, another indication of their dissimilarity is found in the observation that, although irradiation of glycerol solutions caused formation of a TBA

reactive group with absorption at 535 mμ, irradiated glycerol did not cause browning sensitivity in milk or lactose-amino acid systems.

SUMMARY

Ionizing radiations were found to induce the sensitivity of milk to nonenzymatic browning, even when the milk was heated for only a few minutes at 100° C. Radiation-induced changes of lactose were found to be largely responsible for induced browning sensitivity as well as for the production of thiobarbituric acid reactants.

The inter-reaction of lactose with casein in a model system during irradiation increased the intensity of both reactions.

The precursors of both reactions seemed to be produced by the indirect action of ionizing radiations, and the intensity of these reactions was found to increase with dose. The relative quantity of browning precursors was measured as the increase in optical density of digested milk after heat treatment. This value was taken as an objective indicator of radiochemical changes within the lactose-protein complex of irradiated milk.

Radiation-induced browning was prevented when carbonyls were eliminated from the irradiated system.

Two types of nonenzymatic browning were observed. One of these closely resembled the Maillard type of browning of heated milk and exhibited fluorescence in ultraviolet light. A second radiation-induced sensitivity to caramelization was observed in systems that were free of amino groups. These precursors did not fluoresce after browning.

The formation of furans could not be observed in either type of browning. Irradiation of lactose solutions per se as well as in the presence of amino compounds caused formation of reductone-like substances. The findings on determination of carbonyls suggest that these reductones may be in equilibrium with their dehydro form.

These studies on radiation-induced browning indicate that the formation of labile sugar chains need not necessarily be preceded by the formation of N-glycosides. The formation of labile sugar chains with subsequent dehydration, the formation of reductones, and subsequent browning may be catalyzed by oxidative free radicals.

Research on further characterization of browning precursors in model systems as well as in milk is now in progress.

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A SIMPLE MODIFIED METHOD FOR THE DETERMINATION OF VITAMIN A IN STERILE MILK[1]

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The great interest in sterile milk makes it important to have satisfactory methods for evaluating the nutritive properties of such products. The authors have presented a simplified procedure for the determination of vitamin A in sterile milk by using activated glycerol dichlorohydrin as the color-developing reagent. *Editor.*

Spectrophotometric methods have been used for the determination of vitamin A in milk. In studies at the Illinois Agricultural Experiment Station with sterile milk, the method of Olson *et al. (2)*, was used for fat extraction, but the remainder of the procedure of Oser *et al. (3)* was found unsuitable for sterilized stored milk because the unsaponifiable fraction became turbid in isopropanol.

The new colorimetric method proposed by Neri *(1)* was modified for sterile milk by substituting glycerol dichlorohydrin for antimony trichloride. The fat was diluted with its own volume of chloroform and mixed well, and aliquots of the fat-chloroform solution were used. Instead of antimony trichloride, glycerol-dichlorohydrin was used to develop color *(4, 5)* because the color is stable at least for 8 minutes. The combination of the methods *(1, 2, 4, 5)* required less time than the extraction of the unsaponifiable fraction.

METHOD

Reagents

1. *Aldehyde-free alcohol:* Reflux 95% C_2H_5OH with KOH and Al metal for 6-8 hours and then redistill.
2. *Repurified ethyl ether:* Reflux ether over $SnCl_2$ for 4-6 hours and then distill.
3. *Repurified petroleum ether:* Skellysolve B (SSB)
   a. Shake SSB with concentrated sulfuric acid until acid no longer turns dark.
   b. Wash SSB with water until acid-free.
   c. Dry SSB over $CaCl_2$ and then distill.
4. *Activated glycerol dichlorohydrin:* Obtained from J. B. Shohan Laboratories, 78 Wheeler Point Road, Newark, N. J.
5. *Ammonia:* Reagent grade.
6. *Chloroform:* Reagent grade.
7. *Vitamin A acetate:* 1-g. capsule 10,000 U.S.P. units of Vitamin A.

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Extraction procedure:

1. Add 100 ml. of milk to a 500-ml. separatory funnel.
2. Add 15 ml. of concentrated NH_4OH. Shake.
3. Add 100 ml. of redistilled C_2H_5OH. Shake.
4. Add 100 ml. of peroxide-free ether. Shake vigorously.
5. Add 25 ml. of redistilled SSB. Shake vigorously.
6. Allow to stand for 20 minutes.
7. Draw off lower layer into separatory funnel and re-extract with 25 ml. of ether and 10 ml. of SSB.
8. Let combined extract stand for 30 minutes.
9. Filter combined extract through glass wool into a 500-ml. round-bottom flask, and rinse funnel and glass wool three times with 5-10 ml. of ether each time.
10. Evaporate ether in water bath at 60-70° C. under vacuum by connecting flask to aspirator.
11. Evaporate all ether and pour fat into 100-ml. beaker with about six glass beads.
12. Solidify in iced bath and pour out remaining water and alcohol.
13. Melt fat and place it in vacuum oven at 45-50° C. under 5-10 in. vacuum for about 1 hour and then about 3-4 hours at 20-25 in. vacuum.
14. Pipette fat into a 10-ml. graduated, glass-stoppered cylinder without touching sides of cylinder.
15. Add equal amount of chloroform and mix well.

Development of color:

1. Pipette 1 ml. of fat-chloroform solution and 4 ml. of activated glycerol dichlorohydrin into a 10-ml. graduated, glass-stoppered cylinder.
2. Mix contents by inverting the cylinder 10 times and place it in a water bath at 25° C.
3. Read the color at 500 mμ in a spectrophotometer, using 1 ml. of chloroform and 4 ml. of activated glycerol dichlorohydrin as the blank.

CALCULATION

Construction of standard graph:

1. Dissolve a known amount of vitamin A acetate in chloroform and dilute to known volume of 1-16 γ of vitamin A per milliliter.
2. Develop color in standard solutions and plot concentration versus log per cent transmission and determine the equation.
3. Check every lot of glycerol dichlorohydrin against a known standard of vitamin A before using.
4. Substitute the per cent transmission obtained in the equation $N = \frac{a - \log\%}{b}$

where N is the concentration and a and b are constants calculated by the least square method *(6)*.

5. Multiply the final value by proper dilution factor.

Correction for carotene interference:

1. Read standard carotene solutions (0 – 3.5 γ per milliliter of $CHCl_3$) at 420 mμ, using chloroform as a blank.

2. Read the corresponding value of that carotene concentration which will give a color with glycerol dichlorohydrin at 550 mμ.

3. Construct a standard graph for carotene at 420 mμ and 550 mμ with glycerol dichlorohydrin and determine the equations as for vitamin A.

4. Determine the per cent transmission which would be given by the amount of carotene present in the sample and obtain the equivalent per cent transmission on the 550 mμ graph. Calculate the equivalent amount of vitamin A and subtract the value from the total vitamin A.

In the reported recovery experiments, vitamin A acetate solution in $CHCl_3$ of different concentrations was added to butterfat of known vitamin A content, and the total concentration was designated as calculated vitamin A in micrograms per milliliter (Table 1).

TABLE 1

The recovery of vitamin A from fat-chloroform solutions of known concentration

Experiment No.	Vitamin A in the fat-$CHCl_3$ solution	Vitamin A added	Calculated vitamin A	Determined vitamin A	Recovery
	(γ/ml)	*(γ/ml)*	*(γ/ml)*	*(γ/ml)*	*(%)*
1	2.19	2.61	4.80	4.78	99.58
2	3.76	2.63	6.39	6.04	94.52
3	3.74	2.76	6.50	6.46	99.38
4	2.19	5.07	7.26	6.97	96.01
5	2.19	5.22	7.41	6.98	94.20
6	3.76	5.26	9.02	8.19	90.80
7	3.74	5.51	9.25	8.68	93.84
8	2.19	7.60	9.79	9.39	95.91
9	2.19	7.83	10.02	9.31	92.91
10	3.76	7.89	11.65	10.32	89.58
11	3.74	8.27	12.01	10.86	90.42
12	2.19	10.14	12.33	11.36	94.59
13	2.19	10.44	12.63	11.58	91.69
14	3.76	9.53	13.29	12.62	94.96
15	3.74	11.02	14.76	12.74	86.31
16	2.19	12.67	14.86	13.59	91.45
17	2.19	13.06	15.25	13.80	90.49
18	3.76	13.16	16.92	15.34	90.66
19	2.19	15.20	17.39	14.90	86.68
20	3.74	13.78	17.52	15.34	87.56
21	2.19	17.74	19.93	17.57	88.16
22	2.19	20.27	22.46	19.15	85.26
23	2.19	22.80	24.99	20.68	82.75

Results:

Twenty-three recovery experiments were done (Table 1). The standard deviation of the differences between duplicate determinations in the 23 experiments was ± 0.15 γ per milliliter of solution. The recoveries were satisfactory when a low concentration of vitamin A was used. If the fat contains more vitamin A than was used in constructing the standard graph, then it can be diluted further. In these studies, 1 : 1 ratio was found satisfactory.

SUMMARY

Activated glycerol dichlorohydrin was used as the color-developing reagent instead of antimony trichloride. Saponification and extraction of unsaponifiable procedure was omitted since the fat was directly used after dilution. The time required is less than the method for extracting the unsaponifiable fraction.

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WEIGHT LOSSES OF PACKAGED ICE CREAM AND MELLORINE-TYPE PRODUCTS DURING STORAGE

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The purpose of this study was to obtain information as to (a) possible correlation between the weight loss of packaged frozen desserts, volume loss, and organoleptic scores of the product during storage, (b) factors that affect the amount of weight lost during storage, and (c) an evaluation of the relative importance of these factors. Six different types of fat were used in a mix containing 12% fat, 10% m.s.n.f., 15% sucrose, 0.3% gelatin, and 0.05% emulsifier. Fresh condensed skimmilk supplied the major source of the m.s.n.f. Both a batch and Vogt continuous freezer were used. *Editor.*

Research workers are continually looking for ways to lengthen the storage life of frozen dessert products. At present, ice cream and other frozen desserts must be moved into retail channels in a relatively short time in order to avoid flavor and body defects. One of the more serious defects of frozen desserts is volume loss, often termed shrinkage. Although procedures have been developed by which this defect can be controlled, much remains to be learned about its basic causes.

In this connection an observed direct relationship has been reported between weight losses of packaged ice cream and the body and texture scores of the product during storage *(2)*. In the same report a decrease in the volume of ice cream as it lost weight during storage was observed. In a later paper from the same station *(3)* it was reported that ice cream shrinkage or volume loss resulted from dehydration of the product and that this dehydration was also directly related to the weight loss of ice cream. On the other hand, a statement has been published to the effect that there was no indication of water loss affecting ice cream shrinkage *(4)*.

Preliminary work at Oklahoma A & M College substantiated the observation concerning the relationship between body and texture scores and weight loss. During the course of this work it was also found that weight measurements were very accurate, the maximum variation between duplicate weighings being 0.03%. This is greater accuracy than can be obtained with volume measurements or organoleptic examinations. Therefore, it seemed that weight measurements might be a more accurate technique to use in studying certain flavor and body defects of frozen desserts during storage than would the more direct measurements. In view of these findings and the literature reports, it was thought worth while to study some of the factors which might affect the weight loss of packaged frozen desserts during storage.

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The objectives of this study were to determine: *(a)* if the weight loss of packaged frozen desserts could be correlated with volume loss and organoleptic scores of the product during storage, *(b)* what factors affect the amount of weight lost by packaged frozen desserts during storage, and *(c)* to evaluate, if possible, the relative importance of these factors.

PROCEDURE

The frozen desserts used in this study were calculated to have the following composition: 12% fat, 10% milk solids-not-fat, 15% sucrose, 0.3% gelatin, and 0.05% emulsifier. Fresh milk from the college herd was separated and the skim-milk was condensed and used in compounding the mixes. Six types of fat of the following descriptions were used during the course of this work: (A) milk fat, as sweet cream, which had been separated from milk of the Oklahoma A & M College dairy herd; (B) butter churned from cream A; (C) vegetable fat, which was mostly cottonseed oil, hydrogenated to a melting point of 44° C.; (D) vegetable fat which was the same as C except that an emulsifier had been added; (E) beef fat which was hydrogenated to a melting point of 45° C.; (F) beef fat which was the same as E except that an emulsifier had been added.

All six types of fat were used in the preliminary trials and in Trials 3 and 4, whereas the samples used in Trials 1, 2, and 5 were ice cream with sweet cream (A) as the source of fat.

All the mixes were pasteurized at 160° F. for 30 minutes in 10-gal. cans partially immersed in water and then homogenized in a Manton-Gaulin homogenizer at 2,500 lb. of pressure. After storage for 24 hours at 40° F., the mixes were frozen. The desserts used to obtain the data in Trial 2 were frozen in a Vogt continuous freezer, and the other samples were frozen in a 10-gal. batch freezer. All frozen products were taken at an overrun of about 80% and placed in U. S. No. 2 pint brick cartons (medium paraffin). Two or three times as many samples as needed were frozen. After hardening at 2° to –10° F. overnight, the samples to be used for the various trials were selected on the basis of uniform weight.

The samples were weighed on a triple-beam balance which had a sensitivity of 0.1 g. Moisture contents were determined by the Mojonnier procedure *(1)*, and the volume of the frozen dessert samples was determined by the displacement method of Trautman and Nickerson *(6)*. The flavor and body and texture scores which appear in this report are the average scores of three judges.

The statistical methods used in analyzing the data from this study were conducted according to the procedures outlined by Snedecor *(5)*.

RESULTS

Five trials were conducted to compare the weight losses and organoleptic scores of frozen desserts during storage. The frozen dessert samples used in these trials were stored in a chest-type home freezer cabinet at 0° to –8° F. At 2- or 4-week intervals, the samples to be tested were removed from the cabinet, weighed, and scored.

There was no apparent relationship between flavor score and weight loss of the frozen dessert samples in these five preliminary trials. However, the corre-

TABLE 1

Comparison of weight losses and body scores of pint frozen dessert samples during storage at 0° to 8° F. Summary of five preliminary trials

Weight loss	Body and texture score decrease
(%)	*(%)*
0.21	0.00
0.35	1.39
0.66	2.09
1.28	2.44
0.38	0.00
0.86	0.00
1.41	0.70
2.40	5.92
0.38	0.00
0.89	0.00
1.34	0.70
2.68	5.92
0.27	0.00
0.40	0.00
0.80	0.70
1.77	5.92
0.14	1.72
0.30	2.59
0.48	1.72
0.67	2.59
0.90	3.45
1.07	5.17

lation coefficient for body and texture score decrease and weight loss was 0.717 when all five trials were combined (Table 1). This value was statistically significant at the 1% level ($P < 0.01$), and the 95% confidence limits on it were from 0.423 to 0.875.

In the preliminary trials all samples were stored at the same temperature. In view of this, Trial 1 was set up to determine the effect of two different storage temperatures on weight losses and on body and texture scores of pint ice cream packages. In this trial 24 pints of ice cream were paired by weight and one pint of each pair was stored in a freezer cabinet at 4° to 8° F. while the other was stored in the cold room at 2° to −10° F. The stored samples were so arranged that each had the same amount of the carton's surface area exposed to the air.

The results of Trial 1 are shown in Table 2. In this trial the samples that had been stored at 4° to 8° F. were judged coarse or icy after 6 weeks of storage and

TABLE 2

Comparison of weight loss and body score of pint ice cream samples during storage at selected temperatures—Trial 1

Length of storage	2° to −10° F.		4° to 8° F.	
	Weight loss	Body score decrease	Weight loss	Body score decrease
(wk.)	*(%)*	*(%)*	*(%)*	*(%)*
3	0.00	0.00	1.15	1.41
4	0.14	1.06	1.13	4.58
6	0.14	1.06	1.83	4.93
7	0.24	1.06	3.32	4.93
8	0.25	0.70	3.55	8.10

TABLE 3

Weight loss, moisture, and volume comparisons[a] of pint ice cream samples during 20 weeks storage at 1° F. Summary of data—Trial 2

Weeks	Position in freezer cabinet														
	Layer 1—Bottom			Layer 2			Layer 3			Layer 4			Layer 5—Top		
	Wt. loss	H_2O	Vol.	Wt. loss	H_2O	Vol.	Wt. loss	H_2O	Vol.	Wt. loss	H_2O	Vol.	Wt. loss	H_2O	Vol.
	(%)	*(g. per pint)*	*(ml. per pint)*	*(%)*	*(g. per pint)*	*(ml. per pint)*	*(%)*	*(g. per pint)*	*(ml. per pint)*	*(%)*	*(g. per pint)*	*(ml. per pint)*	*(%)*	*(g. per pint)*	*(ml. per pint)*
4	0.24	184.1	443	0.40	186.4	442	0.20	196.6	450	0.25	189.0	459	1.40	190.4	445
8	0.22	184.0	462	0.64	190.9	451	0.34	189.2	455	0.45	189.0	455	2.03	186.7	446
12	0.71	185.0	441	0.68	182.5	451	0.61	194.9	457	0.68	187.2	454	2.62	182.8	444
14	0.73	190.2	447	0.74	193.5	457	0.84	192.9	449	0.67	189.7	453	3.00	181.3	443
16	0.85	193.5	439	0.70	189.2	431	0.60	190.6	445	0.91	195.3	444	3.69	177.9	435
18	0.87	187.7	391	0.77	197.3	456	0.94	201.5	448	1.05	193.6	451	3.95	186.0	438
20	1.06	192.0	450	0.69	197.8	457	1.16	184.0	439	1.15	190.0	451	3.04	181.9	439

[a] Each value represents the average of four pint-pairs.

were thought to be unsalable at the end of 8 weeks. The body score of the samples stored at 2° to −10° F. had decreased very little after 8 weeks. Weight losses were correlated with body scores and a correlation coefficient of 0.692 was obtained for the samples held at 2° to −10° F. and a value of 0.754 was obtained for those held at 4° to 8° F. The number of samples used in this trial was small and the correlation coefficients were not statistically different from zero.

Trial 2 was conducted to determine what relationships exist between weight loss, moisture content, and volume of pint ice cream samples during storage. For this work 400 pints of ice cream were paired by weight. These pint-pairs were then randomized into five layers and four rows in a chest-type home freezer cabinet and stored for 20 weeks. The temperature in all parts of this cabinet was 1° F. ± 1° F. At each sampling period, one pint-pair was removed at random from each row of each layer, making 20 pint-pairs in all. All pints were weighed, then one pint of each pair was used to determine the moisture content and the other to determine volume.

A summary of the data obtained in Trial 2 is shown in Table 3. The correlation between moisture content and weight of the pint samples was high ($P < 0.01$) at each sampling period (Table 4). In addition, the regression coeffi-

TABLE 4

Correlation and regression coefficients by weeks of storage—Trial 2

Correlation				Regression	
Moisture vs. weight		Volume vs. weight		Moisture vs. weight	
(Wk.)	*(Corr. coef.)*	*(Wk.)*	*(Corr. coef.)*	*(Wk.)*	*(b)*
4	0.984	4	0.253	4	0.606
8	0.856	8	−0.149	8	0.590
12	0.979	12	0.553	12	0.669
14	0.985	14	0.443	14	0.659
16	0.983	16	−0.214	16	0.690
18	0.989	18	0.531	18	0.643
20	0.985	20	0.384	20	0.652

cients for moisture vs. weight for these samples were relatively constant through time. The correlation coefficients for volume vs. weight (Table 4) were relatively inconsistent through time. These results were statistically significant ($P < 0.05$) at the 12-, 14-, and 16-week sampling periods but were not statistically different from zero at the other sampling periods.

It is interesting to note that an analysis of variance of these data indicates a statistical difference, significant at the 1% level, due to position or height in the box with layer 5 (the top) losing the most weight. The pint samples in this layer had more carton surface exposed directly to the air than did the samples in the other layers.

For the next two trials, six mixes were compounded with the source of fat as the only variable. After being frozen and hardened, the samples were stored in a chest-type home freezer which was different from the one used in the previous trials and in which there were considerable temperature variations. The average temperature on Side I for package levels 1 (bottom) to 5 (top) consecutively

were 2.0°, 2.0°, 3.5°, 5.5°, and 7.0° F. On Side II the corresponding average temperatures were : 4.0°, 4.5°, 5.5°, 7.0°, and 9.0° F., respectively.

In Trial 3, 12 replicate pints of each of the six different frozen desserts were used. These were stacked against the long walls of the freezer cabinet in three layers; 24 pints per layer with 12 on each side of the cabinet. The samples were weighed at 2-week intervals during a 12-week storage period.

The data obtained in Trials 3 and 4 were evaluated by an analysis of variance technique. In Trial 3 (Table 5) there was a statistical difference, which was significant at the 1% level, in the weight loss of the frozen desserts due to position in the box. Layer 3, the top one, lost the most weight.

TABLE 5

Average per cent weight loss per pint of frozen dessert after 12 weeks storage in a cabinet with variable temperatures. Summary of data—Trial 3

Fat source	Position in freezer		
	Bottom	Middle	Top
(A) Cream	0.37	0.40	2.09
(B) Butter	0.47	0.58	2.71
(C) C.S.O.[a]	1.29	1.15	3.13
(D) C.S.O. & emulsifier	0.34	0.73	2.26
(E) Beef fat	0.39	0.50	2.44
(F) Beef fat & emulsifier	1.24	0.92	3.06
Total for layers	0.68	0.71	2.61

[a] Cottonseed oil.

Since no allowance had been made for temperature variations within the box in Trial 3, another trial (No. 4) was run with frozen desserts which were compounded in the same manner and from the same ingredients as those used in Trial 3. Forty replicates of each treatment or fat source were used in Trial 4. They were stacked along the long walls of the freezer in five layers, or tiers, of 48 pints each, 24 on a side. Each layer was composed of eight random samples of each of the six treatments. The 24 samples of each side in each tier were randomized independently of all other sides and tiers. These samples were weighed at 2-week intervals during the 12-week storage period.

The data obtained in Trial 4 after 12 weeks of storage are summarized in Table 6. In this trial there also was a statistical difference, significant at the 1% level, in the weight loss of the frozen dessert samples which was due to position in the box. The top layer (layer 5) lost the most weight, but the weight losses of the samples in the other layers were not directly related to the temperature in the freezer since layer 4 lost the least weight and layer 2 (bottom) had the second highest weight loss.

There also was a difference, statistically significant at the 1% level, in the weight lost by samples on opposite sides of the cabinet. Differences due to the source of fat were not statistically significant at the 5% level when grouped as individual treatments or as milk fat vs. cottonseed oil vs. beef fat (AB vs. CD vs. EF). In addition, the samples containing fats which had additional emulsifier added to them were not statistically different, at the 5% level, from the samples

TABLE 6

Average per cent weight loss per pint of frozen dessert after 12 weeks storage in a cabinet at variable temperatures. Summary of data—Trial 4

Fat source	Side	Position in freezer box: Bottom 1	2	3	4	(Top) 5	Av. for treatment
(A) Cream	I	1.13	0.80	0.84	0.31	2.48	
	II	1.10	0.78	0.68	0.31	3.05	1.15
(B) Butter	I	1.19	0.87	0.62	0.38	2.59	
	II	1.27	1.07	0.59	0.19	2.12	1.09
(C) Cottonseed oil	I	1.44	0.76	0.90	0.38	2.50	
	II	0.95	0.77	0.31	0.22	2.27	1.05
(D) Cottonseed oil & emulsifier	I	1.13	0.93	0.68	0.60	2.64	
	II	1.16	0.49	0.62	0.44	2.03	1.07
(E) Beef fat	I	1.13	1.15	0.71	0.76	2.32	
	II	1.21	0.77	0.54	0.32	2.12	1.10
(F) Beef fat & emulsifier	I	1.18	1.34	0.81	0.64	3.07	
	II	1.52	0.67	0.44	0.49	2.29	1.24
Av. for sides	I	1.20	0.98	0.76	0.51	2.60	1.21
	II	1.20	0.75	0.53	0.33	2.31	1.03
Av. for layers		1.20	0.87	0.64	0.42	2.46	

containing only the 0.05% emulsifier, which was added to all mixes (ADF vs. BCE).

After Trial 4 had been completed it was found that in handling the samples incident to the weighing procedure the samples on Side I had been warmed to a higher temperature than those on Side II. Accordingly, Trial 5 was set up to determine the effect of these temperature variations on the weight loss of pint ice cream samples during storage.

For Trial 5, 30 pints were selected from a batch of ice cream and divided into three sub-groups on the basis of weight. After hardening, the samples of each sub-group were randomized into two vertical rows, five pints high, and these rows were then placed side by side in the freezer box. At each weighing period (every 2 weeks) one vertical row of each sub-group was left in its original position as a control. The other row, chosen at random, was placed across the cooler from Side I to Side II in a manner designed to duplicate the weighing procedure followed in Trial 4. This was done as follows: The cartons in layer 5 were placed on top of the fifth layer cartons across the cooler and allowed to remain there for 4 hours; layer 4 was placed across the cooler on top of layer 5 and allowed to remain there 3 hours; layer 3 was placed across on top of layer 4 and allowed to remain for 2 hours; layer 2 was placed on top of layer 3 and remained for 1 hours; layer 1 remained in its position at the bottom of the cabinet. After the specified period of time had elapsed, the pint samples were returned to their original position and all samples were weighed. The average temperatures across the cooler in the positions described were: 18° F. for layer 5, 21° F. for layer 4, 22° F. for layer 3, and 25° F. for layer 2.

An analysis of variance of the data obtained in Trial 5 (Table 7) showed no significant differences at the 5% level in the weight losses of warmed samples (those which had been moved) and those of the controls.

TABLE 7

Effect of warming on the average weight loss[a] of pint ice cream samples during 12 weeks of storage in a freezer cabinet at variable temperatures. Summary of data—Trial 5

	Warmed					Control				
	Position in freezer box					Position in freezer box				
Weeks of storage	Layer 1 bottom	Layer 2	Layer 3	Layer 4	Layer 5 top	Layer 1 bottom	Layer 2	Layer 3	Layer 4	Layer 5 top
2	0.15	0.23	0.07	0.07	0.56	0.15	0.31	0.26	0.14	0.61
4	0.29	0.61	0.82	0.41	1.64	0.52	0.78	0.68	0.47	1.63
6	0.36	0.69	0.90	0.47	1.63	0.62	1.16	0.88	0.55	1.81
8	0.49	1.06	1.19	0.61	2.46	0.84	1.38	1.10	0.67	2.49
10	0.69	1.26	1.45	0.72	2.85	0.79	1.74	1.35	0.72	2.77
12	1.15	1.97	1.95	0.80	3.54	1.17	2.21	1.73	0.97	3.40

[a] Each value represents the average per cent weight loss of 3 pints of ice cream.

DISCUSSION AND SUMMARY

In this study there was a positive correlation between decreasing body and texture scores of packaged frozen desserts during storage and the weight losses of the products. In the preliminary trials the correlation coefficient for these variables was 0.717 ($P < 0.01$) and in Trial 1, where a limited number of observations were obtained, correlation coefficients were 0.692 and 0.754. These results appear to substantiate the observations reported in the literature *(2, 3)* concerning the relation between body and texture scores and weight losses of packaged ice cream during storage.

The correlation coefficients for weight vs. moisture content of packaged frozen dessert samples during storage averaged 0.966. Thus, the total variance in weight of these frozen dessert samples could be reduced approximately 93.3% if the moisture content was known. In addition, the regression coefficients for these two variables were relatively constant through a 20-week storage period.

On the other hand, the correlation between weight and volume was low and inconsistent through time. On the basis of these data it does not appear that volume loss could be predicted from weight loss with any degree of accuracy.

The temperature of storage appeared to have some effect on the amount of weight lost by frozen desserts during storage. When the surface area of the carton exposed to the air was constant, the samples stored at higher temperatures lost more weight than did those stored at lower temperatures.

The position of the pint cartons in the freezer box also seemed to have an effect on weight losses of the packaged product. When the temperature of storage was relatively constant the samples in the top layers of the freezer cabinet lost the most weight. These samples had more surface of the carton exposed to the air than did the others.

The position of storage may have had a greater influence on these weight-loss data than did the temperature of storage. When both position and temperature were variables, the top layer of frozen dessert samples lost more weight than did the lower layers. Although the temperature of the samples in each layer differed to some extent in Trials 3 and 4, no constant or logical relationship between temperature and weight loss could be found. It may be that the position

factor overshadowed any differences which might have been caused by temperature in these trials.

Data obtained in this study did not indicate any significant differences, at the 5% level, in the weight losses of frozen dessert samples during storage due to the type of fat in the product.

This report does not necessarily contradict reports of other workers *(2, 3)*, since the frozen dessert samples used in this study were packaged in paraffined cartons that were known to resist shrinkage *(2)*. Reports of other authors often have been based on data obtained from ice cream stored in cartons that contribute to shrinkage *(3)*.

These findings do not rule out possible relationships between shrinkage and the position in the freezer box or the type of fat in the frozen dessert, since the data obtained in this study showed that the correlation between shrinkage and weight loss of pint frozen dessert samples was quite low.

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THE MODE OF ACTION OF ANTIBIOTICS IN THE NUTRITION OF THE DAIRY CALF. I. EFFECT OF TERRAMYCIN ADMINISTERED ORALLY ON THE PERFORMANCE AND INTESTINAL FLORA OF YOUNG DAIRY CALVES[1]

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The need for information on the mechanism(s) involved in the effect of certain antibiotics in stimulating the appetite of nonruminating calves and in causing certain benefits to the animal led to this investigation. Fifteen 3-day-old calves were kept on the experiment for 9 weeks. The results suggest that the presence of enteric streptococci limits the growth potential of young animals. The need for further study to develop improved techniques for determining the extent to which antibiotics affect the intestinal flora of animals is indicated. *Editor.*

It is now well established *(1, 13)* that the feeding of certain antibiotics, such as aureomycin and terramycin, to young nonruminating calves stimulates appetite, increases growth rate, reduces intestinal disturbances, and improves physical appearance. However, there is still a lack of agreement as to the actual mechanism(s) involved. Most of the hypotheses have been challenged by various workers who have been unable to correlate growth stimulation with changes in the intestinal flora.

In view of these divergences of reports and of the paucity of information regarding the effects of antibiotics on the intestinal flora of dairy calves, an experiment was initiated in order to investigate the effect of orally administered terramycin on representative groups of the intestinal flora of dairy calves in an attempt to correlate changes, if any, with the performance of the animals.

This paper is the first of a series of publications on the mode of action of antibiotics in the nutrition of the dairy calf. It is primarily intended to show some of the limitations of standard bacteriological techniques for a study of the mode of action of antibiotics on the flora of the digestive tract of animals.

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[2] This article is part of a dissertation presented to the faculty of the College of Agriculture of Michigan State University in partial fulfillment of requirements for a Master's Degree.

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[4] Present address: Kansas State College, Manhattan.

MATERIAL AND METHODS

Animals used, feeding and management. Fifteen 3-day-old dairy calves were kept on experiment for 9 weeks. The 10 control animals received a ration consisting of whole milk in proportion to body weight and hay and grain fed according to appetite. The five animals in the experimental group received the same basal diet, plus a terramycin supplement[5] added to the milk so as to provide 1 mg. of terramycin per pound of body weight daily.

Bacteriological methods. Fecal samples were collected twice a week directly from the rectum of the calves into sterile Petri dishes. The microscopic method developed by Bortree *et al. (2)* was used to determine the total bacterial count. Lauryl tryptose broth *(10)* was chosen as a presumptive medium to estimate the number of coliforms present. Dextrose azide broth *(12)* was used as an enrichment medium for streptococci; the positive tubes were confirmed by transfer to ethyl violet azide broth *(11)*.

Bacterial sensitivity to antibiotics was determined as follows: single colonies of coliform bacteria were isolated on eosin methylene blue agar plates. Isolation of single colonies of enteric streptococci was performed according to the general procedure established by Sherman *(16)*; in this procedure, the reaction in litmus milk was considered as a test per se and was not used in the identification of the types of streptococci isolated. The standard paper disk method was used for the assays.

During the process of isolation of enteric streptococci for sensitivity assays, some indications were obtained that the metabolism of some of the bacteria living in the intestinal tract of the calves might be affected by feeding terramycin. In order to test this hypothesis, the fermentative activity in sterile skimmilk of several strains of streptococci isolated from the feces of control and terramycin-fed calves was investigated. The strains isolated from the feces of control calves were inoculated each into eight series of 10 test tubes containing 9 ml. of skimmilk and 0 to 50 γ of terramycin per milliliter. The cultures were incubated at 35° C. One tube of each concentration was taken out of the incubator every 6 hours for 48 hours and after 96 hours, and the pH of the skimmilk was determined with a Cenco pH meter. After 24 hours of incubation, one tube of each series was transferred into 10 tubes of skimmilk free from terramycin. A second transfer was made similarly with the 24-hour cultures of the first transfer as the inocula, and pH measurements were taken as previously described. The strains of enteric streptococci isolated from the feces of terramycin-fed calves were inoculated each into a series of 10 tubes of skimmilk free from terramycin. Two successive transfers were also made, and pH measurements were taken according to the procedure and schedule previously described.

Excretion of terramycin. Fecal samples were collected at 0, 6, 9, 12, 15, and 24 hours after the supplement was fed for the first time. The concentration and total amount of terramycin excreted in the urine and in the feces as functions of the intake were also determined. Feces and urine were collected quantitatively

[5] Bi-Con TM-5, guaranteed to contain 5 g. of pure terramycin hydrochloride per pound, was supplied by Charles Pfizer and Co. Inc., Brooklyn 6, N. Y.

every 6 hours for 24 hours at 13 days of age and every 6 hours for 48 hours at 19, 32, and 64 days of age. A modification of the standard paper disk method was used for the assays.

RESULTS

Effects of terramycin on growth rate and feed efficiency. Terramycin feeding significantly increased ($P < 0.01$) the rate of weight gain by 33% above thata of the control animals. The stimulation of growth was greatest during the first 6 weeks. The growth of the control calves was depressed during the first 3 weeks, but their average gain for the first 9 weeks of age was 16% above the Ragsdale standard. The incidence of scours was low and was not affected by the treatment. Appetite and feed efficiency of the terramycin-fed calves were significantly increased above those of the controls. Both appetite and feed efficiency were significantly correlated with the rate of gain.

Bacteriological findings. The results of the total bacterial counts and the number of coliforms and enteric streptococci in the feces of the calves are presented graphically in Figure 1. The total and coliform counts were not significantly different in the control and in the terramycin-fed group. The numbers of the enteric streptococci averaged 162,000 per gram of wet fecal material as

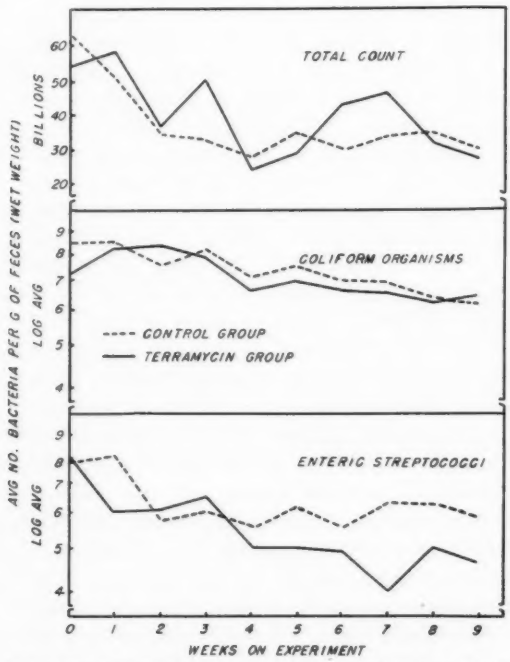

FIG. 1. Effect of feeding terramycin from 3 to 66 days of age on the total bacterial count and the number of coliforms and enteric streptococci present in the feces of dairy calves.

TABLE 1

Effects of terramycin administered orally on the sensitivity to terramycin of strains of coliform bacteria isolated from the feces of young dairy calves

Treatment of the calves	No. of samples	No. of strains	No. of strains resistant to various levels of terramycin		
			500 γ/ml	50 γ/ml	5 γ/ml
Control	13	51	9	10	14
Terramycin	21	65	62	65	65

TABLE 2

Effects of terramycin administered orally on the sensitivity to terramycin of strains of enteric streptococci isolated from the feces of young dairy calves

Treatment of the calves	No. of samples	No. of strains	No. of strains resistant to various levels of terramycin		
			500 γ/ml	50 γ/ml	5 γ/ml
Control	15	62	8	13	16
Terramycin	31	117	60	80	85

compared to 1.44 million per gram in the control group. This difference was found to be significant at the 1% level of probability (the logarithmic averages were used in the statistical analysis). The average enteric streptococci counts for each calf were negatively correlated ($r = -0.63$, significant at the 1% level of probability) with the rate of growth of the animals evaluated in comparison with the Ragsdale standard.

Data pertaining to the studies on sensitivity of coliforms and enteric streptococci to terramycin are summarized in Tables 1 and 2, respectively. With both groups of bacteria, there was a marked increase in the numbers of strains resistant to terramycin when this antibiotic was fed. The values obtained for sensitivity to aureomycin were similar to those obtained with terramycin and therefore are not presented. Coliforms appeared rather sensitive and enteric streptococci highly resistant to dihydrostreptomycin independently of their sensitivity to terramycin and whether or not they were isolated from control or terramycin-fed calves (data not presented).

All but three of the strains of streptococci isolated appeared to be true enterococci, as evidenced by their ability to produce turbidity in brain-heart infusion broth and in tryptose phosphate with 6.5% salt, and to reduce litmus milk. However, the strains isolated from terramycin-fed calves were characterized by a much lower ability to curdle litmus milk, as compared to strains isolated from control calves.

Figure 2 (A and B) illustrates the results obtained in the study concerning the influence of terramycin on cultures of enteric streptococci in skimmilk. Terramycin added to the skimmilk produced a significant decrease in the activity of the strains isolated from the control calves, as measured by acid production. Strains isolated from the feces of terramycin-fed calves followed closely the pattern observed when 50 γ of terramycin per milliliter were added to strains isolated from control calves. No important changes in bacterial activity were

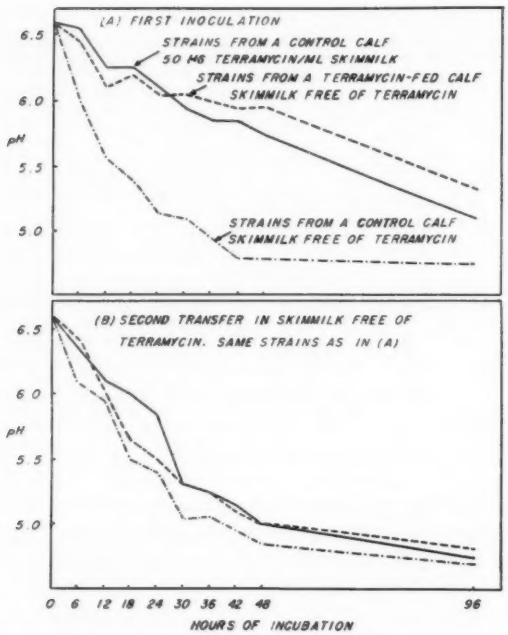

FIG. 2. Fermentative action of strains of enteric streptococci in sterile skimmilk. (A) Terramycin-exposed and control strains of enteric streptococci. (B) Same strains after two transfers in sterile skimmilk free of terramycin.

observed in the first transfer in skimmilk free of the antibiotics, as compared with the results of the initial inoculation. After the second transfer, the influence of the initial addition of terramycin was no longer apparent.

Excretion of terramycin in the feces and the urine. Terramycin was found in the feces 6 to 9 hours after the first administration. The concentrations increased in the following collections. The values obtained after 24 hours ranged from 57 to 250 γ of terramycin per gram of feces on a dry matter basis. The average concentrations of terramycin found in the feces at 13, 19, 32, and 64 days of age were 343, 756, 300, and 121 γ per gram of dry fecal material, respectively. Similarly, the percentages of terramycin intakes excreted were 33, 21, 26, and 21 in the feces and 12, 9, 12, and 4 in the urine at 13, 19, 32, and 64 days of age, respectively. The concentrations of terramycin in the urine varied from 0.7 to 5.2 γ per milliliter.

DISCUSSION

Since the maximum growth response to terramycin in this experiment was obtained during the first few weeks, when the growth rate of the control calves seemed to be depressed, it would appear that terramycin relieved a growth

depression rather than produced a true stimulation of growth. This is in agreement with the hypothesis proposed by Coates *et al. (3)*. However, it should be noted that in the present experiment the incidence of scours was low and was not affected by the treatment. Furthermore, the number of coliform organisms, which is considered among the main causative agents of scours, was not influenced by terramycin feeding.

The results of this investigation would suggest that the presence of enteric streptococci possibly limited the growth potential of the animals since there was a negative correlation between the number of these bacteria in the feces and the growth rate of the calves. If so, part of the growth increase obtained with terramycin in this experiment may have been due to the effect of this antibiotic on enteric streptococci. In a similar and concurrent experiment *(8)*, aureomycin was fed to young dairy calves kept in the same environment as the animals used in the present study. Growth was stimulated approximately to the same extent by aureomycin as by terramycin, but the effect of aureomycin on streptococci was less evident. Voelker and Cason *(18)* and Rusoff *et al. (15)* found no consistent differences in the intestinal flora of dairy calves when fed terramycin or aureomycin.

Similar inconsistencies abound in the literature pertaining to the effect of antibiotics on the intestinal flora of animals. In practically all of these studies, only changes in number of various groups of bacteria have been considered. It is evident that this is only part of the picture; bacterial counts merely indicate an approximation of the number of bacteria in the intestine and give no indication whatsoever concerning their activity. Since terramycin is known to be a bacteriostatic rather than a bactericidal agent when used at low concentrations, the number of the various groups of microorganisms in the feces may not be markedly affected, whereas their growth rate and metabolism may be significantly reduced in some cases or enhanced in others. The effect of terramycin on the ability of enteric streptococci to ferment litmus milk and skimmilk is an example of such changes in the physiology of intestinal bacteria. Of greater significance is the increased resistance of intestinal bacteria to antibiotics after antibiotic feeding; this was demonstrated in the present experiment and has also been reported by others *(4, 5, 6, 7, 9, 14, 17)*. The significance of this increased resistance of bacteria to antibiotics, in relation to the sensitivity of these bacteria to the host defense mechanisms, has been studied and will be presented later.

SUMMARY

An experiment was conducted to investigate the effects of feeding terramycin on the performance and intestinal microflora of dairy calves from 3 days to 9 weeks of age. Terramycin administration increased significantly the rate of weight gain by 33% above that of the control animals. Appetite and feed efficiency were significantly increased. The incidence of scours was low and was not affected by the treatment. Terramycin was detected in the feces 6 to 9 hours after the first feeding. High concentrations of terramycin were found in the

feces during continuous feeding of terramycin and the antibiotic was also present in the urine.

Bacteriological studies revealed no marked difference in total and coliform counts attributable to the feeding of terramycin. However, there was a significant decrease in the number of enteric streptococci when terramycin was administered. Antibiotic sensitivity assays indicated an increase in the number of resistant strains of coliforms and enteric streptococci in the feces of calves fed terramycin. The normal metabolism of enteric streptococci appeared to be impaired by the presence of terramycin in vivo and in vitro. These bacteriological studies indicated that improved techniques should be used to determine the extent to which antibiotics affect the intestinal flora of animals.

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EFFECT OF ADDITION OF INEDIBLE TALLOW TO A CALF STARTER FED TO HOLSTEIN CALVES[1]

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In view of the successful addition of tallow to cattle, sheep, and poultry rations, an experiment was undertaken to test the effect of incorporating various levels of stabilized inedible tallow into a calf starter on feed consumption, growth, and utilization of fat-soluble vitamins, as well as on digestion and mineral balance. The results indicate that inedible tallow can be used in calf starters. *Editor.*

The recent introduction of synthetic detergents has made considerable quantities of inedible animal fats available to the feed industry *(16)*. Information concerning their incorporation in dairy-calf rations and subsequent effect on utilization by the calf is meager.

The early literature on fat utilization by the dairy calf was reviewed in 1942 by Savage and McCay *(34)*. Wider aspects of the problem are found in recent reviews such as those by Deuel *(13)* and Piskur *(28)*. Horton *et al. (20)* have reported an experiment in which 0, 4, or 8.5% stabilized animal fat was added to grain mixtures and fed to lactating dairy cows. The animals consumed the various rations readily, and the added fat had no measurable effect on either milk production, fat content, or the vitamin A content of the milk and blood. Matsushima and Dowe *(24)* found that the addition of unstabilized tallow to the ration of steers reduced the feed required per pound of gain. In this study a vitamin A supplement of 30,000 units per steer per day was not adequate for prevention of hypovitaminosis A symptoms; however, doubling the supplement eliminated the deficiency. Tallow fed to lambs at the 5 or 10% levels gave greater feed efficiency and lower cost of gain than the control ration. A 15%

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level of tallow gave the lowest rate of gain *(21)*. Hogs fed 10 and 15% tallow made more efficient live weight gains, but there was no consistent increase in the rate of gain. The replacement value of tallow for corn averaged 115% *(23)*. Siedler and Schweigert *(36, 37)* found that the addition of choice white grease to chick and dog rations increased feed efficiency and provided a good source of readily available energy.

In view of the successful addition of tallow to cattle, sheep, and poultry rations, this experiment was undertaken to test the effect of incorporating various levels of stabilized inedible tallow into a calf starter on feed consumption, growth, and utilization of fat-soluble vitamins. Because of the reported effects of level of fat in the diet upon digestion and mineral balance *(13)*, these criteria also were investigated.

EXPERIMENTAL PROCEDURE

Animals. Twenty-four Holstein male calves were obtained from the University of Connecticut, Osborn Prison Farm, and Mansfield State Training School and Hospital herds from April through June, 1954. Dams were allowed to nurse their calves for 24 hours or each calf was given 8 lb. colostrum from a frozen colostrum bank. Upon arrival at the research barn each calf was weighed, given 500 mg. of chlortetracycline[2] and 200,000 U.S.P. units of vitamin A from fish oil[3] and placed in an individual tie stall. Each of the first four calves to be received at the barn was assigned to one of four experimental starters containing 0.0, 2.5, 5.0, or 10.0% inedible tallow. Assignment was by random allotment, and this procedure was repeated with each successive group of four calves.

Feeding. The calves were fed herd milk from 2 to 7 days of age at the rate of 9.0% of Ragsdale's weight standards for Holstein female calves at birth *(32)* adjusted to the 0.7 power of the weight. Corresponding values for rate of feeding milk from 8 to 14, 15 to 21, 22 to 28, and 29 to 35 days were 7.5, 6.0, 4.0, and 2.0%, respectively, each adjusted to the 0.7 power of live weight on the day preceding the start of the respective week. From the 8th to 91st day of age, calves were allowed free access to water and artificially dehydrated alfalfa pellets. Starter pellets were fed up to a daily maximum of 4 lb. per calf.

The milk fed was from the University Holstein herd and had an average fat content of 3.3%. It contained 10.2 γ carotenoids and 21.7 γ vitamin A per 100 ml. The artificially dehydrated alfalfa pellets, 3/16 to 5/16 in. in diameter, were purchased on the open market at Kansas City, Mo. The starter pellets, 3/16 in. in diameter, were composed of ingredients listed in Table 1. The tallow was fancy grade (titer $> 41.5°$ C. and free fatty acids $< 4\%$) and stabilized with 8 oz. of Tenox R[4] per ton added at the time of rendering. Chemical analyses of the feeds are shown in Table 1.

[2] Lederle's aureomycin.

[3] The fish oil contained 25% by weight of crude soybean lecithin and 25,000 U.S.P. units of vitamin A per gram and was supplied by the Nopco Chemical Co., Harrison, N. J., through the courtesy of Dr. Melvin Hochberg.

[4] Tenox R, Eastman Chemicals Inc., Knoxville, Tenn. Active ingredients: butylated hydroxyanisole and citric acid.

TABLE 1
Feed composition

	Per cent tallow in starter				Artificially dehydrated alfalfa pellets
	0.0	2.5	5.0	10.0	
Ingredients		*(lb/ton)*			
Tallow	0.0	50.0	100.0	200.0	
Corn meal	499.5	439.5	379.5	257.5	
Soybean meal 44%	280.0	290.0	300.0	322.0	
Oats	300.0	300.0	300.0	300.0	
Wheat bran	200.0	200.0	200.0	200.0	
Linseed meal 36%	280.0	280.0	280.0	280.0	
Dried skimmilk	100.0	100.0	100.0	100.0	
Cane molasses	100.0	100.0	100.0	100.0	
Dehydrated alfalfa meal	200.0	200.0	200.0	200.0	
Di-calcium phosphate	20.0	20.0	20.0	20.0	
Salt	20.0	20.0	20.0	20.0	
Irradiated yeast[a]	0.5	0.5	0.5	0.5	
Total	2000.0	2000.0	2000.0	2000.0	
Chemical composition		*(%)*			
Dry matter	89.11 ± 0.27[b]	89.01 ± 0.32	89.31 ± 0.31	90.23 ± 0.33	90.52 ± 0.42
		(% of dry matter)			
Crude protein	23.72 ± 0.14[b]	23.28 ± 0.13	23.34 ± 0.13	22.89 ± 0.13	22.11 ± 0.18
Ether extract	3.59 ± 0.07	6.10 ± 0.21	8.60 ± 0.18	12.74 ± 0.23	4.12 ± 0.13
Nitrogen-free extract	57.00 ± 0.37	55.44 ± 0.56	52.67 ± 0.03	49.43 ± 0.32	40.21 ± 0.39
Crude fiber	7.16 ± 0.11	7.65 ± 0.04	7.08 ± 0.06	6.74 ± 0.06	21.04 ± 0.23
Ash	8.53 ± 0.08	8.13 ± 0.09	8.31 ± 0.16	8.20 ± 0.07	12.52 ± 0.18
Calcium	0.76 ± 0.03	0.89 ± 0.19	0.74 ± 0.03	0.77 ± 0.03	1.74 ± 0.03
Magnesium	0.28 ± 0.01	0.35 ± 0.03	0.28 ± 0.01	0.26 ± 0.01	0.22 ± 0.01
Phosphorus	0.74 ± 0.06	0.95 ± 0.11	0.81 ± 0.03	0.83 ± 0.02	0.26 ± 0.01
		(mg/lb)			
Carotene	3.51 ± 0.20	3.39 ± 0.20	3.45 ± 0.12	3.68 ± 0.13	45.15 ± 17.27
		(% of dry matter)			
Total digestible nutrients	78.44	81.72	84.22	89.02	68.21
		(therms/100 lb. of dry matter)			
Estimated net energy	75.23	80.00	83.63	90.61	60.36

[a] Standard Brands type 27-F containing 12 million I.U. of vit. D/lb.
[b] Arithmetic mean plus and minus its standard error.

On each calf's 92nd day of age, it was placed on a vitamin A depletion ration *(14)* for the purpose of estimating body stores of vitamin A *(41)*. The first 16 calves remained on the vitamin A depletion ration until each calf's blood plasma vitamin A level decreased to less than 4 γ/100 ml. for two consecutive weeks, at which time the calf was slaughtered. The remaining eight calves were fed the vitamin A depletion ration for only 2 weeks to estimate depletion time *(41)*. At the end of this 2-week period, each calf was fed a mixture of equal parts of dehydrated alfalfa pellets and starter pellets at the rate of 2.5 lb. of mixture per 100 lb. live weight. The mixture fed each calf contained the same starter it had received previously, and this regime was maintained for 21 days. After 7 days each calf was placed in a metabolism stall for quantitative collection of urine and feces from the 15th through the 21st day, to estimate digestibility coefficients and mineral and nitrogen balances.

To supplement the calf digestion and mineral balance data, four ram Shropshire lambs were subjected to a digestion trial in a 4 × 4 balanced Latin square design with change-over feeding. They were fed mixtures of equal parts chopped field-cured and field-baled alfalfa hay and the previously described starters. The lambs averaged 222 days of age and 84 lb. in live weight at the beginning of the experiment. The chopped alfalfa hay graded U. S. No. 1 Alfalfa Light Timothy Mixed Hay and consisted of 3.3% foreign material and 87.4% alfalfa, 11.5% timothy, and 1.1% clover. The average proximate composition of this hay, expressed as per cent of dry matter, was: crude protein, 18.93; ether extract, 2.07; nitrogen-free extract, 44.53; crude fiber, 24.88; and ash, 9.59. The rate of feeding was 2.6 lb. of mixture per 100 lb. of initial live weight adjusted at the end of each of the 3-week periods for an anticipated weekly increase of 1 lb. in live weight.

Health. All calves were observed at least three times daily throughout the experiment for scours, bloat, and other disorders. Scours, when accompanied by rectal temperatures of 103° F. or above, was treated with an intravenous injection of a 25% solution of sulfamethazine[5] at the rate of 26.3 ml. per 100 lb. live weight, followed by oral administration of sulfamethazine at the rate of 6.5 g. per 100 lb. live weight for two additional days. Bloat was relieved by stomach tube when an animal became tympanitic.

One calf on the 10% level was lost on the 63rd day of the feeding trial from an occlusion of the esophagus, and one calf on the 0.0% level died of bloat before collections could be made during the metabolism study. Missing values for these calves were calculated *(12)*.

Criteria and analyses. Feeds, fed and refused, were weighed to the nearest 0.1 lb. At 28-day intervals samples of feed were analyzed for proximate constituents *(2)* and carotene content by a modification of the method of Moore and Ely *(26)* by employing MgO rather than $CaPHO_4 \cdot 2H_2O$ for the chromatographic separation. Total digestible nutrients (TDN) were calculated according to the method of Schneider *et al. (35)* for feeds on which there are few or no data on digestibility for cattle. Estimated net energy (ENE) was calculated from the TDN values by the formula of Moore *et al. (27)* which utilized Forbes' data.

Live weights were recorded upon arrival, at 7 days of age, and at weekly intervals thereafter. Measurements of height at withers, heart girth, and girth of paunch were taken at 35 days of age and at 28-day intervals thereafter.

Venous blood samples were drawn at 7 days of age and then at biweekly intervals for hemoglobin *(15)*, plasma carotenoids and vitamin A *(3, 22)*, and plasma tocopherol *(29, 30)* determinations. Vitamin A depletion time was the number of weeks required for a calf's plasma vitamin A to decrease to 4 γ/100 ml. During depletion, blood samples were taken at 7-day intervals for the above criteria.

Prior to slaughter of the 16 calves, spinal fluid pressures were obtained by puncture of the subarachnoid space *(40)*. At slaughter, tissue samples from the

[5] Lederle's Sulmet.

supraspinatus, long head of triceps, *biceps femoris*, and *gastrocnemius* muscles were obtained for creatine analysis *(33)*. The entire liver was ground and samples were taken for carotenoid and vitamin A determinations *(10)*. Total liver tocopherols were estimated by a modification *(9)* of the method of Quaife and Harris *(31)* except that chromatography *(39)* on a properly prepared Floridin column *(7)* rather than selective hydrogenation of the molecular distillate was employed to remove carotenoids and vitamin A *(31)*. Liver lipids were determined during the course of the tocopherol analysis *(31)*.

Feces and urine were quantitatively collected during the last 7 days of each calf's 21-day period and similarly for each of the lambs' four 21-day periods. Calcium, phosphorus, magnesium, and nitrogen contents of the feeds and feces samples and urinary nitrogen were determined by standard A.O.A.C. procedures *(2)*. After wet-ashing the urine *(18)*, calcium and magnesium were determined volumetrically by the method of Cheng *et al. (11)* and phosphorus colorimetrically by the method of Fiske and Subbarow *(17)*.

Standard statistical procedures were followed in the analyses of the data *(12, 38)*. The treatment sums of squares with 3 degrees of freedom were subdivided into a no-tallow versus added-tallow contrast, and into an among-levels of added-tallow contrast. The sums of squares attributable to among-added levels of tallow were further subdivided into linear and quadratic trends on the logarithm of the level of tallow in the starter.

RESULTS AND DISCUSSION

Feed consumption and growth. The addition of tallow did not affect palatability. All the calves, except one, consumed the maximum amount of starter allowed during the latter portion of the feeding trial. One calf on the 2.5% level preferred dehydrated alfalfa pellets rather than starter. This phenomenon of individual preference of roughage has been previously observed *(14)*.

TABLE 2

Effect of different levels of tallow in a starter on feed consumption of Holstein calves

Criterion	Per cent tallow in starter				Standard deviation of a calf
	0.0	2.5	5.0	10.0	
	(total lb/calf)[a]				
Milk	143.0	141.9	146.8	143.2	11.0
Starter	220.7	209.4	220.2	204.2	49.1
Artificially dehydrated alfalfa pellets	44.6	77.5	64.1	74.3	52.1
Dry matter	254.6	274.0	272.8	269.1	36.6
Total digestible nutrients	212.1	230.1	236.2	239.4	28.0
	(total therms/calf)[a]				
Estimated net energy	207.6	225.9	235.1	241.7	27.2
	log [(γ carotene/lb live weight/calf/day)][a]				
Carotene intake	2.29	2.41	2.38	2.49	0.20
	(geometric mean γ carotene/lb live weight/calf/day)				
	195	257	240	309	—

[a] Data from the 7th through the 91st day of age.

There was no appreciable difference among treatments in the amount of starter consumed (Table 2). The calves receiving tallow appeared to consume more roughage, but this tendency was masked by large individual variations within groups. Calves that received tallow in the starter consumed on the average more TDN and more ENE ($P < 0.10$) than the average calf fed the starter containing no tallow. The carotene intakes reflected the average consumption of dehydrated alfalfa pellets.

The increased intake of TDN and ENE resulted in a 5 to 6% increase in growth for all levels of tallow (Table 3). No differences in height at withers were apparent among calves fed the starters containing different levels of tal-

TABLE 3

Effect of different levels of tallow in a starter on growth and feed utilization of Holstein calves

Criterion	Per cent tallow in starter				Standard deviation of a calf
	0.0	2.5	5.0	10.0	
Live weight			*(lb.)*		
Initial at 7 d. of age	102	100	106	102	11
Increase to 91 d. of age	109	115	114	116	20
Height at withers			*(in.)*		
At 91 d. of age	35.46	35.12	35.21	35.50	1.30
Heart girth					
At 91 d. of age	38.71	39.42	39.33	39.75	1.99
Girth of paunch					
At 91 d. of age	47.00	51.33	49.71	49.45	4.52
Feed utilization	*(lb. dry matter intake/lb live weight increase)*				
Dry matter	2.42	2.40	2.38	2.31	0.25
	(lb. TDN intake/lb live weight increase)				
Total digestible nutrients	2.03	2.00	2.06	2.06	0.20
	(therms ENE/lb live weight increase)				
Estimated net energy	2.00	1.96	2.05	2.07	0.18

low, whereas heart girth and girth of paunch were slightly greater in the calves receiving tallow. Since heart girth reflects condition of the calf and girth of paunch reflects roughage consumption, these two criteria apparently agreed with the feed consumption data.

Inclusion of tallow in the starter appeared to decrease the amount of dry matter required per pound increase in live weight (Table 3). This result was not statistically significant, but the trend agreed with results obtained in other studies *(21, 23, 24)*. The utilization of TDN and ENE was not affected by the addition of a high source of energy in the ration. However, because of the narrow range in live weight increases as well as in TDN and ENE intakes, a significant change in utilization of these estimates of nutritive intake could hardly have been expected.

Blood constituents. Inappreciable differences were observed in the hemoglobin, plasma carotenoid and plasma vitamin A values during the feeding of the starters containing different levels of tallow (Table 4) and during the feed-

TABLE 4

Effect of different levels of tallow in a starter on some blood constituents of Holstein calves

Criterion	Per cent tallow in starter				Standard deviation of a calf
	0.0	2.5	5.0	10.0	
Hemoglobin			*(g/100 ml.)*		
Initial[a]	11.85	12.93	12.22	10.91	1.95
Average[b]	10.12	11.14	10.59	10.46	1.24
Average adjusted for initial	10.19	10.65	10.46	11.01	0.76
Plasma carotenoids			*(γ/100 ml.)*		
Initial[a]	22	16	17	19	6
Average[b]	38	57	46	57	23
Average adjusted for log carotene intake	48	53	45	48	14
Plasma vitamin A					
Initial[a]	14.4	14.1	13.9	15.1	4.2
Average[b]	18.4	16.2	16.6	16.9	2.9
Average adjusted for log carotene intake	17.9	16.3	16.6	17.5	2.8
Plasma tocopherol			*(mg/100 ml.)*		
Initial[a]	0.16	0.15	0.15	0.20	0.05
Average[b]	0.15	0.16	0.16	0.20	0.05
Average adjusted for initial	0.16	0.17	0.16	0.22	0.04

[a] Value at 7 d. of age.
[b] Av. of 6 biweekly values 21 to 91 d. of age.

TABLE 5

Effect of previous feeding of different levels of tallow on some blood constituents and live weight changes and vitamin A depletion time during feeding of a vitamin A depletion ration to Holstein calves

Criterion	Per cent tallow in starter				Standard deviation of a calf
	0.0	2.5	5.0	10.0	
			Weekly rate of change		
Blood constituents[a]			*(g/100 ml.)*		
Hemoglobin	0.37	0.02	0.23	0.06	0.56
			(γ/100 ml.)		
Plasma carotenoids	−6.59	−9.71	−6.42	−10.72	12.00
Plasma vitamin A	−4.72	−2.30	−2.48	−2.78	3.88
			(mg/100 ml.)		
Plasma tocopherol	0.01	0.01	−0.01	−0.02	0.04
			(lb.)		
Live weight[a]	14.4	14.4	13.3	14.3	3.2
Vitamin A depletion time[b]			*(Weeks)*		
Average	3.8	4.7	4.6	4.3	1.4
Average adjusted for log carotene intake	4.2	4.6	4.6	3.9	1.2

[a] Values for 4 replicates.
[b] Values observed for first 4 replicates and calculated values *(41)* for 2 replicates from blood values observed during the 2-week depletion period.

ing of the vitamin A depletion ration (Table 5). When the plasma carotenoids and vitamin A values of the feeding period, as well as the vitamin A depletion time, were adjusted to the logarithm of the daily carotene intake expressed as micrograms per pound live weight *(1)*, it was readily apparent that the ingested carotene was utilized equally by all dietary groups.

Total plasma tocopherols tended to be higher in calves fed the starters containing tallow (Table 4). This was possibly due to a greater intake of tocopherol from these starters and/or to the greater consumption of the dehydrated alfalfa pellets. Because of the difficulties inherent in the analysis for tocopherols in feedstuffs containing wheat by-products *(8)*, no attempt was made to analyze the feeds for tocopherols. Therefore, no quantitative estimates can be made as to the effect of the inclusion of tallow in calf starters on tocopherol utilization by the dairy calf.

Slaughter data at the terminaton of the vitamin A depletion period. Spinal fluid pressures, expressed as logarithms, averaged 2.17 (148 mm. H_2O, geometric mean). For liver tissue the following average values were obtained: carotenoids, 22 γ/100 g.; vitamin A, 21 γ/100 g.; total tocopherols, 0.34 mg/100 g.; and liver lipids, 3.76 g/100 g. These criteria were not affected by levels of tallow in the starter and had standard deviations per calf of 0.09, 6, 33, 0.09, and 0.27, respectively.

The average creatine (total creatinine) content of the *supraspinatus*, long head of the triceps, *biceps femoris*, and *gastrocnemius* were, respectively, in milligrams per 100 g., 454, 492, 512, and 508 with standard deviations of a calf of 16, 36, 38, and 42.

Allowing the calves a maximum of 4 lb. of starter reduced their roughage consumption at this age and resulted in the comparatively short vitamin A depletion period. The muscle creatine values appear to be within the "normal" ranges as observed by Blaxter *et al. (5)*. Although the liver tocopherol values were slightly lower than the "normal" values given by Blaxter *et al. (4)*, the differences in analytical procedures, molecular distillation used in this study, and no molecular distillation used by Blaxter *et al.* might possibly explain the differences in the magnitude of these values.

Health. Calves had, on the average, 3.0 days with scours during the feeding phase of this experiment. Treatments with sulfamethazine, initiated only when the condition was accompanied by temperatures greater than 103° F., averaged 0.2 per calf. These criteria appeared to be affected inappreciably by level of tallow in the starter. Incidence of bloat appeared to be unusually severe in the calves fed the 0.0, 2.5, and 5.0% levels of tallow in the starter, averaging four, eight, and four observations per calf, respectively, for these groups. Calves fed the 10.0% level of tallow in the starter exhibited no bloat. Possibly tallow fed in sufficient quantities results in lowered surface tension and less foaming in the rumen.

Apparent digestibility of rations and mineral and nitrogen balances. The inclusion of tallow in the starter of calves resulted in slightly lower apparent digestibility of dry matter ($P < 0.05$), organic matter ($P < 0.05$), crude protein

TABLE 6

Effect of different levels of tallow in a starter on apparent digestibility coefficients of calves and lambs

Criterion		Per cent tallow in starter				Standard deviation
		0.0	2.5	5.0	10.0	
		(%)				
Dry matter	Calves	73.2	69.4	69.1	67.9	1.2[a]
	Lambs	71.6	72.8	72.2	70.3	2.4[b]
Organic matter	Calves	78.4	70.3	71.4	70.2	2.1
	Lambs	74.4	75.4	74.9	73.8	1.8
Crude protein	Calves	79.8	75.5	74.2	72.9	2.8
	Lambs	78.5	78.7	79.0	78.5	1.0
Ether extract	Calves	60.7	62.8	71.9	80.4	1.3
	Lambs	70.8	80.6	80.2	87.0	3.9
Crude fiber	Calves	36.0	36.9	38.3	36.5	1.4
	Lambs	51.9	50.4	53.2	51.6	5.6
Nitrogen-free extract	Calves	81.1	78.3	76.2	75.8	1.4
	Lambs	80.0	80.9	80.1	77.3	3.0

[a] Standard deviation of a calf.
[b] Standard deviation of a lamb's observation per period.

($P < 0.20$), and nitrogen-free extract ($P < 0.10$), and in marked increases in apparent digestibility of ether extract ($P < 0.01$), Table 6. Although the coefficients of digestibility for the lambs were, with the exception of crude fiber, of approximately the same magnitude for the above criteria, only the ether extract coefficient exhibited appreciable change with the inclusion of tallow in the starter. In this case, as with the calves, the coefficients were considerably higher ($P < 0.01$) when tallow was included in the starter. The depression in apparent digestibility of dry matter, organic matter, crude protein, and nitrogen-free extract with increases in added tallow in the calf rations may have been due to the tallow having acted as a readily available source of energy. Similar results have been observed with added corn oil or lard *(6)* and glucose *(19, 25)*. Another possible explanation is the change in nutritive ratios, which increased from approximately 1:3 to 1:4 with the additions of tallow. There is no ready explanation for the lack of depression of apparent digestibility of these constituents in the sheep rations or for the marked differences between species in crude fiber digestion. It should be realized that the two trials are not strictly comparable, having been conducted at different times, with different species, and with different roughages. No attempt was made to estimate fecal soaps *(13)*, which may explain the marked increases in apparent digestibility of the ether extract in both trials.

In connection with the mineral studies, only fecal calcium, in the study with the calves, was significantly increased by inclusion of tallow in the starter ($P < 0.10$). The fecal calcium excretion in g/calf/day was 13.7, 16.0, 19.2, and 26.1, respectively, for the 0.0, 2.5, 5.0, and 10.0% levels of tallow with a standard deviation per calf of 2.0. Average daily retention of calcium, magnesium, and phosphorus was, for the calves and lambs, respectively, 17.6, 7.2; 2.5, 0.8; and

7.0, 0.6. The precision of these values expressed as standard deviations per calf and per lamb per period was respectively, 6.3, 1.4; 0.2, 0.4; and 1.1, 1.3. Average daily retention of nitrogen, which was unaffected by level of tallow in the starter, was for the calves and lambs, respectively, 35.6 and 6.0, with standard deviations per calf and per lamb per period of 7.0 and 1.3.

SUMMARY

Twenty-four male Holstein calves were fed limited whole milk to 35 days of age, a maximum of 4 lb. per day of calf starter containing 0.0, 2.5, 5.0, and 10.0% inedible stabilized tallow, and ad libitum alfalfa pellets to 92 days of age. The calves fed starters containing tallow consumed more calculated total digestible nutrients, which resulted in 5 to 6% greater increases in growth. The pounds of dry matter required per pound of live weight increase were less in the calves fed starters containing tallow, and total digestible nutrient utilization was not appreciably affected. Based on blood plasma values during the above feeding period and during a subsequent vitamin A depletion period, carotene and tocopherol utilization was unaffected by the inclusion of tallow in the starter. Digestibility and mineral balance studies with eight of the above calves and with four lambs indicated that when tallow was fed, there was a decrease in the apparent digestibility of dry matter, organic matter, crude protein, and nitrogen-free extract in the calves, whereas ether extract was increased for both species. Fecal calcium increased with the inclusion of tallow in the ration of calves. The results of this study indicate that inedible stabilized tallow could be used in calf starters.

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RELATION BETWEEN AGE OF CALF, BLOOD GLUCOSE, BLOOD AND RUMEN LEVELS OF VOLATILE FATTY ACIDS, AND IN VITRO CELLULOSE DIGESTION[1,2]

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This study adds to our knowledge of the development and function of the rumens of young calves as they increase in age, as well as the metabolic utilization of the products resulting from microbial fermentation in the rumen. Specific information was obtained on the levels of blood glucose and volatile fatty acids; the rumen juice concentration of reducing substances and volatile fatty acids; the in vitro cellulose digestion; and the relationships existing between these factors. Six male Holstein calves were utilized in each of three trials. *Editor.*

Changes that occur in the development and function of the rumen of the young calf as it increases in age are not well understood. In addition, more information is needed on the metabolic utilization of the products resulting from microbial fermentation in the rumen. It has been well established that the concentration of glucose in the blood is higher in the young calf than in the mature ruminant and that there is a subsequent decline in this metabolite as the calf ages. Apparently, there is a change in energy metabolism from the state of glucose being the major energy metabolite to a system in which the short chain acids serve to meet a large portion of the energy needs. Many investigators relate this decline to the development of the rumen *(4, 5, 6, 11, 15)* but others *(8, 9)* believe that a different explanation is involved. Reid *(13)* reports that a large proportion of the decline in whole blood glucose was due to a steady and almost complete disappearance of glucose from the corpuscles. Concurrent with advancing age, increases in blood concentration of volatile fatty acids in sheep have been noted by Reid and by Craine and Hansen *(5)*, working with goats. Conrad *et al. (3)* found that in calves the volatile fatty acids in rumen fluid increased from 60 meq. per liter at 4 weeks to 92 meq. at 9 weeks of age. The increasing ability of the calf to utilize cellulose has been used as a criterion of rumen activity. Conrad *et al. (4)* and Armstrong *et al. (1)* employed cellulose digestion trials with calves, and Lodge and associates *(10)* used an in vitro technique.

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[2] Taken from a thesis presented by R. D. McCarthy to the Graduate School of the Pennsylvania State University in partial fulfillment of the requirements for a Master of Science degree.

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The objectives of this study were to ascertain the changes occurring in the early life of calves in *(a)* the levels of blood glucose and volatile fatty acids; *(b)* the rumen juice concentration of reducing substances and volatile fatty acids; *(c)* the in vitro cellulose digestion; and *(d)* the relationships existing between these factors.

EXPERIMENTAL PROCEDURE

Six male Holstein calves were utilized in each of three trials, two of which were conducted concurrently. All calves were allowed to remain with their dams and receive colostrum for at least 2 days. By 5 days of age all animals had been moved to the experimental barn and placed on trial. The only major difference between trials was in the rations supplied. The calves in Trials 1 and 2 received 5 lb. of whole herd milk twice daily until they were weaned at 42 days of age. The calves in Trial 3 received milk replacement for the same period of time. Hay was fed ad lib. to all the calves. The animals in Trial 1 received excellent quality, second-cutting alfalfa and the calves in Trials 2 and 3 received a fair quality mixed hay. Calf starter was fed to appetite until a maximum daily intake of 5 lb. was reached. The starter used in Trial 1 had a calculated 17% digestible protein and 70% TDN, whereas that used in Trials 2 and 3 was calculated at 12.3% digestible protein and 71% TDN. All animals were housed in solid-walled, individual pens located in an artificially lighted and ventilated barn. The bedding for the calves consisted of wood shavings.

Both blood and rumen juice samples were collected when the calves were 1 week of age and every week thereafter until 15 weeks of age. Samples were taken approximately 3 hours after the morning feeding. Seventy ml. of blood were drawn from the jugular vein at the time of sampling, potassium oxalate being used as the anticoagulant. Shortly after the blood was obtained, rumen juice samples were taken by means of a stomach tube. The collected juice was immediately strained through two layers of cheesecloth and then placed in a heated box for transport to the laboratory, where a 50-ml. portion was introduced into an artificial rumen. Protein-free filtrates then were made on both blood and the remaining portion of rumen juice by the addition of 0.3 *N* barium hydroxide, 5% zinc sulfate, and sufficient distilled water to make the required dilutions.

Determinations of glucose in blood and reducing substances in rumen juice were made by the Somogyi colorimetric method *(14)*. The concentration of total volatile fatty acids in blood and rumen juice was determined by steam distillation of the protein-free filtrates, followed by titration with sodium ethylate with bromothymol blue as the indicator. After corrections for the blanks and a small loss due to incomplete recovery of the volatile acids, as determined by use of standard solutions, the total acids were calculated as acetic.

The ability of rumen microorganisms to digest cellulose was measured by employing an artificial rumen technique similar to that described by Burroughs *et al. (2)*. The anthrone method *(7)* was used for the determination of cellulose, in this case, ground filter paper. Per cent digestion was calculated on the basis

FIG. 1

of a series of blanks, which were put through the entire procedure with the exception that rumen juice was not added.

RESULTS[4]

During the first 5 weeks the blood glucose values (Figure 1) for Trial 1 showed a steady decline. Trial 2 showed fluctuating values but did tend to decline. However, the glucose values for Trial 3, the replacement-fed calves, began at a much lower level and did not decrease during this period. Following the marked decline into the adult ruminant range at 6 weeks all trials showed some weekly variations but exhibited essentially the same pattern. From 6 weeks through the duration of the experimental period the values for all three trials remained in the adult ruminant range of 40 to 60 mg. per 100 ml.

[4] Throughout the presentation of results the values reported for each trial are the mean values for six calves.

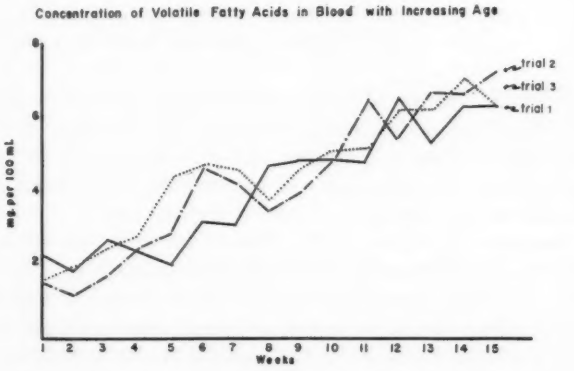

FIG. 2

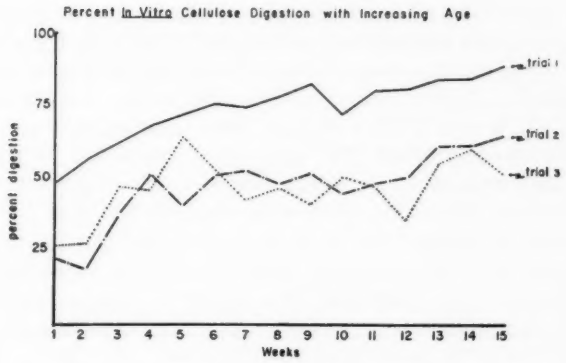

FIG. 3

The concentration of volatile fatty acids in blood as related to age is shown in Figure 2. All trials exhibited a definite trend to increase throughout the entire experimental period. In both Trials 2 and 3 there appeared to be a more rapid increase in volatile fatty acid levels during the first 7 weeks than in Trial 1. In addition, the replacement-fed calves (Trial 3) had higher values during the first 6 weeks than did the milk-fed calves of Trial 2. From 8 weeks through the end of the period little difference between trials was noticeable. The average level for all 18 calves gradually increased from 1.78 mg. per 100 ml. at 1 week to 6.53 mg. per 100 ml. at 15 weeks of age.

Per cent in vitro cellulose digestion for all three trials is presented in Figure 3. It is at once apparent that the per cent digestion was much greater in Trial 1 than in Trials 2 and 3. This can be explained by the fact that the time of incubation in Trial 1 was 48 hours but in Trials 2 and 3 it was reduced to 24 hours. During the course of Trials 2 and 3 a number of rumen juice samples from calves of several ages were run in duplicate artificial rumen jars,

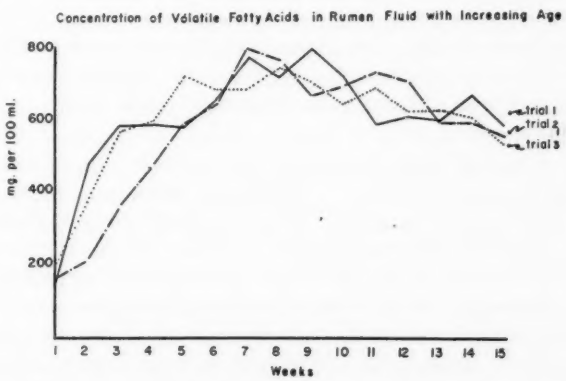

FIG. 4

one being incubated 24 hours and the other 48 hours. The additional 24 hours of incubation increased considerably the amount of cellulose digested, the average difference being 38.1 percentage points. If this increase were applied to the amounts digested in Trials 2 and 3, they would be very similar to the values obtained in Trial 1. For Trial 1 the per cent digestion increased throughout the entire experimental period. The same trend was shown in Trials 2 and 3 even though the values were lower and tended to show more fluctuations. In all trials the most rapid increase occurred during the first 5 or 6 weeks.

Concentration of volatile fatty acids in rumen juice is plotted against age in Figure 4. Here again the similarity between trials in following the general pattern is striking. All trials showed a rapid increase in concentration of volatile fatty acids until 6 or 7 weeks of age. The concentration remained at this plateau of 700 to 750 mg.% until 10 or 11 weeks, when the levels of volatile acids declined somewhat, to around 600 mg.%. The replacement-fed calves of Trial 3 reached a higher concentration more rapidly than did the calves in the other trials.

A separate analysis of variance was conducted on the data obtained concerning each factor measured in a given trial. The analysis took into account the values obtained for individual calves during each of the 15 weeks. The variance due to calves and that due to weeks were determined in each instance. In every trial the decline in blood glucose that occurred with increasing age was significant at the 1% level. Likewise, the increase in blood volatile fatty acids was highly significant in each trial. Increases in rumen juice volatile acids and per cent in vitro cellulose digestion were highly significant in all trials.

A graph is not presented for reducing substances in rumen juice because the values showed such large fluctuations between weeks, and especially between calves during the same week, that little information could be obtained from this part of the study.

From the graphs it is apparent that some differences existed between trials during the first 6 weeks. To check this apparent difference, Trials 2 and 3 were compared during this period. Both trials were conducted during the early spring of 1955 and the calves received the same fair quality mixed hay and the same calf starter. Management and sampling techniques were identical. The only difference between trials was that the calves in Trial 2 received whole herd milk for the first 6 weeks whereas the calves in Trial 3 received milk replacement for the same period.

The replacement-fed calves (Trial 3) had a lower concentration of blood glucose during the first 6 weeks, a difference which was highly significant statistically. Trial 3 calves had higher levels of volatile fatty acids both in blood and in rumen juice, the difference being significant at the 1% level in rumen fluid and the 5% level in blood. Although not statistically significant, cellulose digestion was greater in the calves that received milk replacement. The average over the 6-week period was 36.1% digestion for calves fed milk and 40.0% for the calves in Trial 3. Greater gains in body weight were registered by the calves of Trial 3, which gained 43.8 lb. over initial weight during the 6 weeks as

TABLE 1

The statistical correlations between the various factors measured in all three trials

Correlations	Coefficients of correlation		
	Trials		
	I	II	III
VFA in rumen juice and % cellulose digestion	0.690**	0.712**	0.662**
Blood glucose and blood VFA	−0.666**	−0.835**	−0.639*
Blood glucose and % cellulose digestion	−0.897**	−0.870**	−0.418
Blood VFA and % cellulose digestion	0.809**	0.834**	0.647**
VFA in rumen juice and blood glucose	−0.799**	−0.776**	−0.499
VFA in rumen juice and blood VFA	0.405	0.636*	0.543*
Reducing substances in rumen juice and VFA in rumen juice	0.437	0.420	0.623*
Reducing substances in rumen juice and % cellulose digestion	0.106	−0.016	0.710**
Reducing substances in rumen juice and blood glucose	−0.435	0.146	0.094
Reducing substances in rumen juice and blood VFA	0.189	−0.196	0.192

* Significant at the 5% level.
** Significant at the 1% level.

compared to an average gain of 40.0 lb. for the calves of Trial 2. This difference was significant at the 5% level. Thus it appears that the feeding of milk replacement effected a more rapid transition to adult-like levels of metabolites.

In order to study the relationship between blood glucose, blood volatile fatty acids, rumen fluid volatile fatty acids, rumen juice reducing substances and in vitro cellulose digestion, coefficients of correlation were obtained. These coefficients are presented in Table 1. Comparisons were based on the values for the given factors by weeks and were computed for each trial. It is apparent that the relationship between the various factors was similar in the milk-fed calves (Trials 1 and 2); however, in Trial 3 there were some exceptions. Table 1 points out two facts: first, correlations between factors other than reducing substances in rumen juice were highly significant during the transitional period in the life of a calf; second, a difference between milk-fed and replacement-fed calves, as has already been discussed.

DISCUSSION

The results obtained in this experiment clearly indicate a transition, as the young calf matures, in the level of metabolites involved in energy metabolism. At birth and for a short time afterward the calf is apparently relying on glucose for its major energy needs. This is indicated by the high levels of glucose and the low levels of short chain acids in the blood during this early period. As indicated by these trials, this is not a static condition; rather gradual changes begin to occur very early in life. Blood glucose values begin to decline while the concentration of volatile fatty acids in the blood increases. At the same time rumen activity is increasing, as indicated by the rising levels of volatile acids in the rumen fluid and the increase in per cent cellulose digestion. Statistically, the changes that occur in these factors were highly correlated; however, although these correlations show that these changes occur at the same time, they cannot be construed as proof of the relationship. There is no indication of whether these factors are related by some controlling mechanism or if they influence each other.

It appears likely that the increase in concentration of volatile fatty acids in the blood is a direct result of increased rumen activity. In this work, as per cent cellulose digestion increased so did volatile fatty acid levels of rumen fluid and also the concentration of volatile acids in blood. The levels of acids in the blood did not increase at the same rate as the levels of these volatile acids in rumen juice. Three possible explanations for this are: first, the concentration of volatile fatty acids was measured in venous blood, and consequently a portion of the acids may have been metabolized by the tissues and removed before the blood sample was collected; second, even though the concentration of volatile acids in the rumen is high, the rumen itself may not yet be of sufficient size that the total amount produced can adequately meet the needs of the calf. Third, the rumen epithelium may require some development before the volatile fatty acids can be absorbed at the maximum rate.

The decline in blood glucose with increasing age cannot be as readily explained. Some workers, McCandles and Dye *(11)*, Craine and Hansen *(5)*, and Hibbs *et al. (6)*, suggested that the decrease may result from increasing rumen activity. Others, Jacobson *et al. (8)*, Lambert *et al. (9)* and *Reid (13)*, believe that there is some other explanation for the decline in blood glucose. In the research reported here blood glucose declined gradually for the first 5 weeks. The values fell sharply after the discontinuance of milk feeding at 6 weeks of age. One possible explanation for the large decline in blood glucose has been suggested by the report of Murley *et al. (12)*. During the milk feeding period, when samples were collected 3 hours after feeding, a temporary alimentary hyperglycemia may have existed. However, after the milk was removed from the diet, the condition was not present and the result was therefore an accentuation of the decline. This does not explain the gradual decline in glucose values but is offered only as a possible reason for the sharp fall between 5 and 6 weeks of age. The factors that cause blood glucose levels to decline have not been clearly shown.

It is apparent from the work reported here that the transition in energy metabolism begins very early in the life of a calf. All the factors measured began to shift toward the adult values at 1 week of age. Indications that the young calf is at least a partially functioning ruminant are the levels of volatile fatty acids in the rumen fluid and the ability of this fluid to digest cellulose at 1 week of age. The most rapid increases in per cent cellulose digestion and the concentration of volatile fatty acids in rumen juice occur during the first 6 weeks. Likewise, blood volatile fatty acids at 6 weeks of age are at a level where they could make a considerable contribution to the energy needs of the animal. Under the conditions of this research it could be said that, although rumen activity was not completely developed, it could be considered to be similar to that of the mature animal by 5 or 6 weeks of age.

SUMMARY

Blood and rumen juice were collected from 18 male Holstein calves at 1 week of age and every week thereafter until 15 weeks of age. Results of the

analyses show a lowering of blood glucose levels concurrent with increasing levels of blood volatile fatty acids. At the same time, rumen juice volatile fatty acids and per cent in vitro cellulose digestion increased as the calves advanced in age. The significance of these findings and the possible relationship between these factors are discussed.

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THE EFFECT OF AGE OF THE CALF ON THE AVAILABILITY OF NUTRIENTS IN VEGETABLE MILK-REPLACER RATIONS[1]

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The study was designed to determine the influence of age on the ability of the calf to digest vegetable milk-replacer rations by determining the amounts of nitrogen, calcium, and phosphorus that were retained at four ages (10, 18, 26, and 34 days). Whole milk and evaporated milk were fed as standards of comparison. Male Holstein calves 2 to 4 days of age were assigned to the experiment at random. The milk replacers were not satisfactorily utilized until the calves were approximately 25 days of age. *Editor.*

There is a scarcity of data relevant to the digestion of vegetable milk replacers by the young calf, particularly with respect to the effect of age. A previous investigation in the use of vegetable milk replacers in calf nutrition indicated that weight gains and feed consumption were unsatisfactory until the calves were approximately 25 days of age *(15)*. Earlier studies by Shaw *et al. (18)* demonstrated that 4- to 7-day-old calves were able to digest only one-fifth of the starch consumed, but by 3 to 4 weeks of age the calves were able to digest over 90% of the starch. A study conducted by Archibald *(1)* on raw and cooked calf meals indicated that 9-week-old calves were able to digest feed almost as well as adult cattle. Digestion studies on whole milk have been conducted with calves *(3, 16)*, pigs *(21)*, and rats *(14)*. In all cases the whole milk was highly digestible.

This experiment was designed to ascertain the influence of age on the ability of the calf to digest vegetable milk-replacer rations and, by balance trials, to determine the amount of nitrogen, calcium, and phosphorus retained at four ages. Whole milk and evaporated milk were fed as standards of comparison.

EXPERIMENTAL PROCEDURE

Experimental design. A 4 × 4 Latin square metabolism experiment was conducted by using 4-day preliminary periods and 4-day collection periods. The

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sequence of the experimental periods and age of calves at each collection period are shown in Table 1.

Selection of animals. Male Holstein calves were obtained when 2 to 4 days of age and assigned to the experiment at random. Any calf that was unable to complete the experiment, owing to either poor appetite or sickness, was replaced for the entire experiment by the next available calf.

Feeding and management. The calves were kept in individual pens on heavy metal screens during the preliminary period to prevent the ingestion of bedding. The pens were heated with overhead heat lamps. Whole milk was fed from open pails at the rate of 4 lb. twice daily as soon as the calves would drink.

TABLE 1

Experimental design showing the age of calves and rations fed

Period	Age	Calf No. C922	C923	C925	C926
	(days)	*(Ration)*			
1	10-14	W.M.[a]	Repl. 7	Evap.	Repl. 8
2	18-22	Repl. 7	Repl. 8	W.M.	Evap.
3	26-30	Evap.	W.M.	Repl. 8	Repl. 7
4	34-38	Repl. 8	Evap.	Repl. 7	W.M.

[a] Abbreviations: W.M., whole milk; Repl., milk replacer; Evap., evaporated milk.

TABLE 2

Composition of daily rations

Ration	Amount fed: Whole milk	Evaporated milk	Water	Milk replacer
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*
Whole milk	8	–	–	–
Evaporated milk	–	4	4	–
Milk replacer	–	2	6	0.5

TABLE 3

Composition of the milk replacers

Ingredients	Milk replacer 7	8
	(%)	*(%)*
Ground corn	57.4	53.4
Soybean flour (52.4%)	40.0	34.0
Distillers dried solubles	—	10.0
Steamed bonemeal	1.5	1.5
Calcium carbonate	0.5	0.5
Salt	0.5	0.5
Vitamin and trace mineral mixture[a]	0.1	0.1
Total protein	24.31	23.75

[a] Mixture contained:

Vitamin A concentrate 20,000 U.S.P. units/g	20 g.
Irradiated yeast 9,000 units vitamin D/g	5 g.
Cobaltous sulfate ($CoSO_4 \cdot 7H_2O$)	3 g.
Cupric sulfate ($CuSO_4 \cdot 5H_2O$)	2 g.
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	11 g

The calves were fed at the same time each morning and evening throughout the experiment. On the sixth day of age the calves were placed on the experimental ration for the first period. The composition of the rations and the amounts fed are presented in Table 2.

Feed formulation. The composition of the milk-replacer rations is shown in Table 3. The corn and distillers dried solubles were finely ground to facilitate the dispersion in the milk-water mixture. The trace ingredients were premixed before being added to the final mixture. The mixed feeds were stored in metal containers to prevent contamination.

Fecal and urine collection. Fecal collections were made beginning on the tenth day of age with $8 \times 3 \times 15$ in. pliofilm bags *(7)*. The bags were prepared with cellulose tape as shown in Figure 1 A. The placing of the long strips of

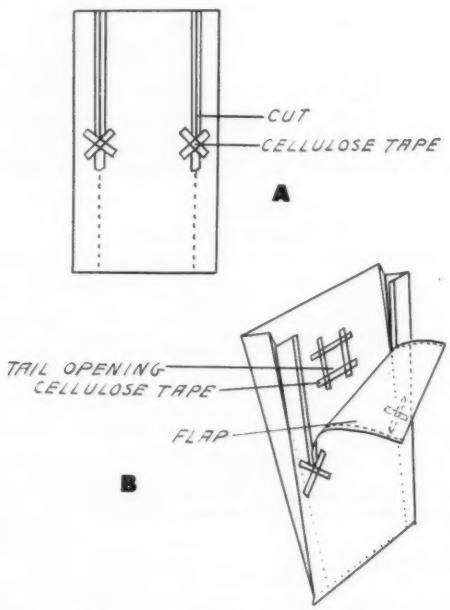

FIG. 1. A. Preparation of pliofilm bag before cutting the flap. B. Shows the bag ready for use after the tail opening and flap have been cut.

cellulose tape was followed by the shorter crosspieces to prevent tearing when the long tapes were cut to form the flap. After the bag was taped and cut as indicated in Figures 1A and B, the tail opening was made by cutting a reinforced square hole, the approximate size of the tail.

The entire area back of the hip bones and flank of the calf was clipped. The underside, posterior to the prepuce, also was clipped. A web strap, 1½ in. wide

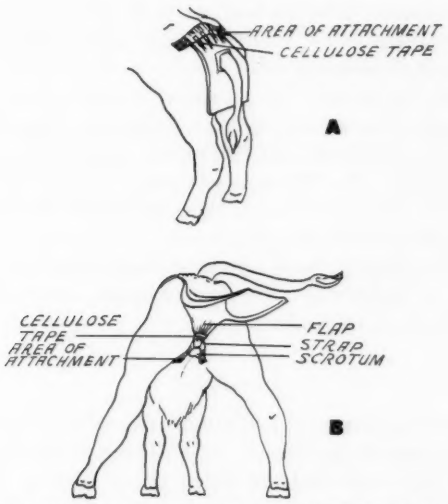

FIG. 2. A. Shows the position of top strap and attached pliofilm bag. B. Shows the position of lower strap and attached pliofilm bag.

and approximately 9 in. long, was attached to the rump of the calf midway between the hip and pin bones (Figure 2 A) with branding cement[5] *(9)*. The strap on the underside of the calf was attached with branding cement as shown in Figure 2 B.

The prepared pliofilm bag was attached by passing the tail through the tail opening and passing the end of the bag under the back strap, folding it over the strap and taping down with strips of cellulose tape (Figure 2 A). The lower flap (Figure 2 B) was attached by passing the flap between the lower strap and the body, folding back and winding with cellulose tape. Care was used to prevent undue strain on the bag. The calves were tended four times daily and the bag was changed at these times if feces had been voided. The bag was removed by cutting with scissors.

After removal, the bag and contents were weighed, the bag was cut open and the contents were placed in a glass jar. The feces for the entire period were refrigerated during the trial and dried immediately after the collection of the last sample.

During the collection periods the calves were kept in metal metabolism cages[6] 36 in. wide, 30 in. high, and 48 in. deep. The calves stood on a heavy coarse-mesh screen with a finer screen and a urine funnel and drain below. The two screens and funnel were removable.

The urine was collected in glass bottles containing 20 ml. of concentrated hydrochloric acid. Samples were refrigerated until analyzed.

[5] Product of Nebraska Salesbook Co., Lincoln, Neb.

[6] Product of George H. Wahmann Mfg. Co., Baltimore, Md.

Analytical procedures. The dry matter, crude fiber, nitrogen, ether extract, ash, calcium, and phosphorus content of the milk replacers and feces were determined by A.O.A.C. methods *(2)*.

Milk: Nitrogen (Kjeldahl), fat (Babcock), total solids (Mojonnier), wet ash for calcium and phosphorus *(4)* with selenium oxychloride as a catalyst, calcium (modification of Shohl and Pedley, *19)* and phosphorus (modification of Fiske and Subbarow, *8)*. The nitrogen, ash, calcium, and phosphorus procedures for the urine were the same as those indicated for the milk.

Statistical analyses: Snedecor *(20)*.

Calculation: The coefficients of apparent digestibility of the milk replacers were determined by difference. Nitrogen, calcium, and phosphorus balances were calculated for all of the rations.

RESULTS

The coefficients of apparent digestibility of each of the feeds are presented in Table 4. The apparent dry matter digestibility of the raw whole milk was high, averaging 94.8%, with little variation due to period. That of evaporated milk was low during the first period but reached its maximum value during the second period. The milk replacer digestibilities were low the first period,

TABLE 4

Coefficients of apparent digestibility of the feeds by the individual calves and the average daily retention of nitrogen, calcium, and phosphorus for each period

Period	Calf	Digestion coefficients					Retention		
		DM	CP	EE	CF	NFE	N	Ca	P
		(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(g.)*	*(g.)*	*(g.)*
					Whole milk				
1	C922	92.5	81.2	96.5	—	98.2	6.79	4.01	2.63
2	C925	96.2	92.8	97.9	—	98.0	6.62	3.87	1.72
3	C923	95.0	93.2	98.6	—	95.2	7.03	2.94	1.87
4	C926	95.6	93.0	98.0	—	96.8	9.04	3.75	2.26
Av.		94.8	90.1	97.8	—	97.1	7.37	3.64	2.12
					Evaporated milk				
1	C925	76.8	56.5	72.3	—	97.4	−4.27	0.99	−0.28
2	C926	91.1	80.7	96.5	—	95.0	4.52	2.92	1.60
3	C922	87.9	82.7	87.3	—	94.4	3.95	1.38	0.81
4	C923	91.1	87.8	96.1	—	92.9	8.59	1.67	1.35
Av.		86.7	76.9	88.1	—	94.9	3.20	1.74	0.87
					Milk replacer 7				
1	C923	33.8	−8.5	−1.0	−0.2	58.9	−3.57	1.16	0.35
2	C922	50.2	1.7	48.9	−3.9	76.8	−1.28	1.24	0.14
3	C926	76.3	56.3	128.6	−8.0	84.0	2.92	2.34	1.32
4	C925	76.0	51.6	100.2	−1.5	89.5	1.66	1.42	0.61
Av.		59.1	25.3	69.2	−3.4	77.3	−0.07	1.54	0.61
					Milk replacer 8				
1	C926	16.3	−44.5	−110.9	0.0	56.8	−7.96	1.31	−0.12
2	C923	54.8	15.7	−7.5	7.6	83.9	−6.08	0.95	0.10
3	C925	78.1	54.9	124.8	13.5	87.7	1.55	2.22	0.97
4	C922	71.2	44.8	89.7	3.8	84.8	3.26	1.91	1.03
Av.		55.1	17.7	24.0	6.2	78.3	−2.31	1.60	0.50

higher the second period, and attained their maximum value in the third period (26 to 30 days of age). The coefficients for the dry matter in the two milk replacers averaged 25.0, 52.5, 77.2, and 73.6, respectively, for the four successive periods.

The apparent digestibilities of the crude protein in the raw whole milk were 81.2, 92.8, 93.2, and 93.0%, respectively, for the four successive periods. The corresponding values obtained for the evaporated milk were 56.5, 80.7, 82.7, and 87.8%. The values for the two milk replacers averaged –26.5, 8.7, 55.6, and 48.2%.

The nitrogen-free extract of the raw whole milk and that of the evaporated milk were 97.1 and 94.9% digestible, respectively, with little variation due to period. In comparison, the digestibility of the nitrogen-free extract in the two milk replacers for the four successive experimental periods averaged 57.9, 80.4, 85.9, and 87.2%, respectively.

TABLE 5
Analyses of variance of the digestibility and balance data

Source of variation	df	F values					
		DM	CP	NFE	N	Ca	P
Periods	3	4.29	5.08[a]	2.14	5.03[a]	0.28	0.90
Animals	3	0.83	0.61	1.37	0.28	1.21	0.48
Rations	3	8.94[a]	12.14[b]	6.26[a]	7.44[a]	9.28[a]	5.12[a]
Error	6						

[a] Significant at the 5% level of probability.
[b] Significant at the 1% level of probability.

Analyses of variance (Table 5) indicate that the variance in apparent digestibility among feeds was significant for dry matter and nitrogen-free extract and highly significant for crude protein. The variance among animals was not significant for any measures. The apparent digestibility of crude protein was significant among periods and highly significant among rations.

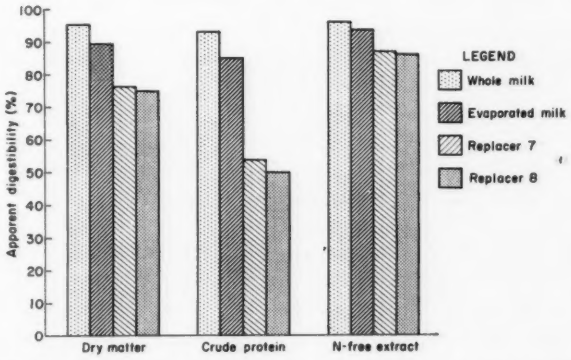

FIG. 3. Shows the coefficients of digestibility of dry matter, crude protein and nitrogen-free extract in raw whole milk, evaporated milk and the milk-replacer rations by 26- to 38-day-old calves.

The average apparent coefficients for the third and fourth periods for the dry matter, crude protein, and nitrogen-free extract of the four feeds are presented in Figure 3. Very little difference was found between the two milk replacers. The evaporated milk was slightly less digestible than the raw whole milk, but the crude protein in the milk replacers was considerably less digestible than that of either milk.

The nitrogen balance was negative during the first period of evaporated milk feeding and during each of the first two periods when the milk replacers were fed (Table 4). Calcium retention was significantly greater for calves fed raw whole milk than for those fed the evaporated milk or the milk-replacer rations. Calves fed raw whole milk retained an average of 81.7% of their calcium intake compared to an average of 40.3% for the evaporated milk and 39.0% for the two milk-replacer rations. Phosphorus retention averaged 60.9% for the whole milk ration compared to 25.5% in calves receiving evaporated milk and 16.5% in those receiving the two milk-replacer rations. Low or negative phosphorus balances were observed during each of the first two periods on the milk-replacer rations.

Analyses of variance of the nitrogen, calcium, and phosphorus balance data indicate significant differences among rations, but only among periods for the nitrogen balance (Table 5).

DISCUSSION

The technique described for the collection of feces satisfied the requirements of this investigation when 4-day collection periods were used. A preliminary experiment indicated that 2-day fecal and urine collection periods were of insufficient length when the milk replacers were fed. Very little difficulty was encountered when the feces were costive and when the bags were changed four times daily.

The average coefficients of apparent digestion found in this experiment for the dry matter, ether extract, and nitrogen-free extract of raw whole milk are similar to those obtained for pigs *(21)*, 7½- to 8-month-old calves *(11)*, 1- to 17-day-old calves *(16)*, and 5- to 14-day-old calves *(3)*. The mean coefficient of dry matter digestibility for the raw whole milk was also in close agreement with the coefficient obtained in a preliminary experiment. The average value of 90.1% for the crude protein digestibility of raw whole milk is lower than the values cited by some investigators *(3, 11, 21)*. An inspection of Table 4 reveals that the average value is due to the low apparent digestibility of the crude protein during the first period (10 to 14 days). Parrish *et al. (16)* also reported low crude protein digestibilities for colostrum and whole milk in calves between 3 and 8 days of age.

The data indicate that the evaporated milk was less digestible than the raw whole milk under the conditions of this experimnet. The largest difference in the digestibility of the nutrients in whole milk and evaporated milk occurred between the crude protein fractions of the two milks. Lower digestibility values for evaporated milk were also obtained in preliminary experiments with two

different lots of milk. A significant decrease in the nutritive value of milk proteins when milk was heat-treated has been observed in rats *(5, 6, 10, 12, 14)*. In contrast, Whitnah *(22)*, using the rat nitrogen balance method, and Schroeder *et al. (17)*, using the dog nitrogen balance method, reported that the nutritive value of the protein in evaporated milk did not differ significantly from that of raw whole milk. However, the differences observed in this investigation may be attributable in part to the type and degree of heat treatment and possibly to the physiological age of the animals used. The differences obtained between the evaporated milk and raw whole milk suggest that young calves, at the ages used, may not be physiologically equivalent to the animals used by the latter investigators.

Digestibility values for the milk replacers were lower than those for the milks. The crude protein in the milk replacers was considerably less digestible than that in either milk, but less difference occurred in the nitrogen-free extract portion. Crude fiber in the milk-replacer rations appeared to be indigestible throughout the experiment and manifested little evidence of increasing in digestibility with increased age of the calves within the limits of this investigation. The ether extract values for the milk replacers are of questionable value because of the small amount of fat present in the milk replacers as compared to the milks.

The digestibility of raw whole milk varied little with age of the calf. In contrast, evaporated milk was less digestible by the 10- to 14-day-old calf than by the 18- to 22-day-old calf. The milk replacers were not utilized satisfactorily until the calves were approximately 25 days of age. This is in agreement with a previous finding *(15)* and with results reported by Shaw *et al. (18)* in their studies of starch digestion in the young calf. The increased utilization of feed by the older calves may be due to a physiological change, such as increased enzyme activity, in the alimentary tract.

The data show that in 26- to 38-day-old calves the apparent digestibilities of raw whole and evaporated milk are similar for dry matter, crude protein, and nitrogen-free extract. The milk replacers were almost identical, but significantly less digestible than the milks. The coefficients of digestibility for the crude protein in the milk replacers were considerably lower, dry matter was slightly higher, and the nitrogen-free extract was noticeably higher than those reported by Archibald *(1)*, who fed calf meals to 9-week-old calves. This suggests that crude protein of vegetable origin is less digestible by 26- to 38-day-old calves than by 9-week-old calves. The dissimilarities cited could be due, in part, to differences in the composition of the rations fed.

The average daily excretion of urinary nitrogen was similar for all rations; however, fecal nitrogen averaged 1.8 g. on the raw whole milk ration, 3.8 g. on the evaporated milk ration, and 8.9 g. on the milk-replacer rations. Since the dry matter intake was similar on all rations, the metabolic fecal nitrogen would be expected to vary only slightly, although some increase in metabolic fecal nitrogen may occur owing to a difference in the physical state of the milk replacers as compared to that of the milks. The increased fecal nitrogen observed with replacer

feeding may represent undigested protein not assimilated by the calf, possibly due to a lack of sufficient or appropriate enzymes needed for splitting the protein molecules. The presence of undigested protein in the feces of young calves fed milk replacers could account, in part, for the "putrefactive" nature of the feces noted previously *(15)*. The decreased digestibility of crude protein and increased fecal nitrogen of calves fed evaporated milk may have been due to the adverse effect of heat on the milk proteins during processing, as emphasized by Melnick and Oser *(13)*.

The greater calcium and phosphorus retention by calves fed raw whole milk than by those fed the evaporated milk or milk-replacer rations indicates that the calcium and phosphorus of raw whole milk are more efficiently utilized by the young calf.

The data indicate that raw whole milk is readily digested and utilized by the young calf. Apparently the young calf is not able to digest and assimilate evaporated milk as readily as raw whole milk although the differences are minor. The data also show that the milk replacers were of little nutritional value to the calf until it was approximately 25 days of age; thereafter, these feeds were considerably more digestible.

SUMMARY

Four male calves were assigned to a 4×4 Latin square metabolism experiment to determine the apparent digestibility of two milk replacers, evaporated milk, and raw whole milk at 10, 18, 26, and 34 days of age. Fecal collections were made by utilizing a simplified bag technique. Nitrogen, calcium, and phosphorus balances were also determined.

The mean coefficients of apparent digestion of raw whole milk were: dry matter, 94.8; crude protein, 90.1; ether extract, 97.8; and nitrogen-free extract, 97.1. The dry matter of evaporated milk was 76.8% digested by a 10- to 14-day-old calf and an average of 90.0% by 19- to 38-day-old calves. The average apparent dry matter digestibility of the milk replacers increased from 25.0% for 10- to 14-day-old calves to an average of 75.4% for 26- to 38-day-old calves. The most digestible fraction was the nitrogen-free extract and the least digestible, the crude protein.

The crude protein of the raw whole milk was slightly less digestible by the 10- to 14-day-old calf than in subsequent periods; the maximum value for evaporated milk was not reached until the calf was 19 to 22 days of age. The crude protein of the milk replacers was markedly less digestible than that of either of the milks during the first collection period and did not reach the maximum value until the calf was 26 to 39 days of age.

The amount of nitrogen, calcium, and phosphorus retained was greater for calves fed raw whole milk than for those fed the evaporated or milk-replacer rations. Nitrogen and phosphorus balances were low or negative in 10- to 22-day-old calves fed the milk-replacer rations, but the older calves had positive balances.

The milk replacers were not satisfactorily utilized until the calf was approximately 25 days of age.

ACKNOWLEDGMENTS

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A HYALIN-FIBRIN COMPLEX IN THE BOVINE MAMMARY GLAND[1,2]

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The authors report results of their efforts to determine the composition and structure of intra-alveolar bodies in the udder and to relate the conditions under which they were found. Tissue sections from 25 dairy-cow udders were taken as soon as possible after slaughter. The bodies found in 15 of the udders are described. *Editor.*

Intra-alveolar bodies in bovine mammary glands have been previously reported in histological studies. Ottolenghi *(11)* first mentioned the presence of these bodies in 1901 and considered them to behave like a true amyloid. McFadyean *(9)* published a photomicrographic study of the so-called amyloid bodies of the cow's udder, describing the bodies as consisting of a complex nucleus surrounded by a circular stratified structure showing radial markings. McFadyean concluded that these bodies could not be considered amyloid for any reason other than the stratified structure, which resembled certain starch grains and corpora amylacea of the prostate. A similarity to urinary calculi was noted. Zimmerman *(13)* suggested a lipoid composition, and Joest and Steck *(7)* suggested that albumen was essential in their make-up. Hadwen and Gwatkin *(4)* proposed that they were calcium deposits on the basis of analyses which indicated the presence of a mixture of dicalcium and monocalcium phosphate.

This paper reports the results of efforts to characterize the composition and structure of intra-alveolar bodies in the udder and to relate the conditions under which they were found.

METHODS AND PROCEDURE

A microscopic study was made on tissue sections (7 μ) from 25 dairy-cow udders. The tissue samples were obtained as soon as possible after slaughter. One sample was taken from each quarter vertically above the teat about midway between the dorsal and ventral perimeters of the udder. Each sample was fixed with Bouin's fluid and prepared for sectioning by infiltration with paraffin *(3)*. Harris' hematoxylin-eosin and Mallory's triple connective tissue stains were used along with differential staining procedures *(3, 6)* to test for the presence of amyloid, hyalin, fibrin, calcium, and mucin. The tissues were further tested

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[2] Data presented in this paper are from a thesis submitted by the senior author to the graduate faculty of Purdue University in partial fulfillment of the requirements for the Master of Science degree.

[3] Present address: U. S. Air Force.

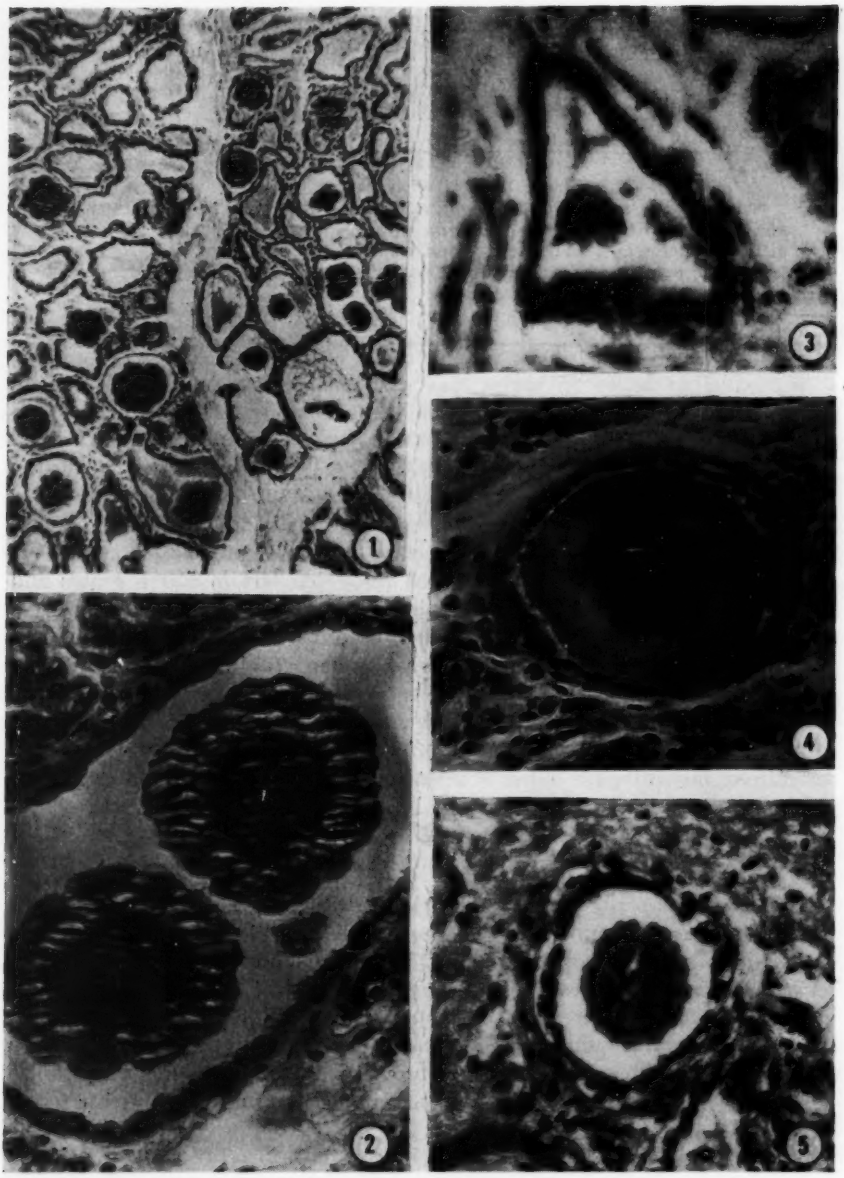

FIG. 1. Distribution of the intra-alveolar bodies found in the cow's udder. ×125.

FIG. 2. Intra-alveolar bodies consisting of a network of fibrils. ×544.

FIG. 3. An aggregation of leucocytes bound together with fibrin. ×544.

FIG. 4. An intra-alveolar body occupying the entire lumen of the alveolus. ×544.

FIG. 5. An intra-alveolar body showing laminations, radial striations, and varying affinity for strain. ×544.

for mucin by subjecting the tissues to a series of solutions of NaOH ranging from 0.1 *N* to 2 *N*. Jenkin's decalcifying fluid and 3% HNO_3 were used to detect calcium. Photomicrographs were made of the intra-alveolar inclusions.

RESULTS AND DISCUSSION

Structure and composition. Microscopic examinations revealed the presence of peculiar bodies lying free in the alveoli and ducts of some dairy-cow udders. These bodies generally occurred singly; however, in some instances there were as many as three per alveolus. Although their frequency varied from section to section, they were generally dispersed rather uniformly throughout the section. A typical example of the distribution and location of these bodies in the mammary gland tissue is presented in Figure 1.

Although all bodies were circular in nature, two structural types were observed. Some consisted of a meshed network of fibrils or thin strands (Figure 2), and others had a dark hematoxylin-stained nucleus with a lighter eosin-stained envelope showing concentric laminations and sometimes fine radial striations (Figures 4 and 5). The dark round mass in Figure 3 appeared to be made up primarily of an aggregation of leucocytes with a collection of fibrils around it. The body may occupy the entire lumen and exert a compressing effect on the epithelium (Figure 4). The radial striations and the varying affinity for the stain between layers are shown in Figure 5.

The outer stratified area of the hyalin-fibrin body was semi-translucent, homogeneous in appearance, and regularly stained like simple hyalin (bright red with carbolfuchsin, and blue-black with Weigert's fibrin stain). Nuclei of bodies of this type gave a positive reaction for blood fibrin as did the entire body when the structure consisted of a meshed network of fibrils. The bodies did not develop the true amyloid red color with methyl violet, or blue with Lugol's iodine solution and sulfuric acid. They failed to develop the opaque black indicative of calcium when treated with $AgNO_3$. The bodies remained completely unaltered after immersion of tissue sections in Jenkin's decalcifying agent and 3% nitric acid for periods of 1 to 48 hours. The series of solutions of NaOH, ranging from 0.1 *N* to 2 *N*, had no effect and they did not stain magenta or the pale lilac color of mucin with carbolfuchsin. A summary of the reaction to various differential staining procedures is given in Table 1.

TABLE 1

The reaction of the intra-alveolar bodies to the various differential staining procedures

Stain	Differentiates	Reaction	Results
Iodine and H_2SO_4 (5-10%)	Amyloid	Red to blue in a few minutes	Negative
Methyl violet	Amyloid	Violet red	Negative
Weigert's fibrin stain	Hyalin	Blue-black	Positive
Carbolfuchsin (Russell)	Hyalin	Bright red	Positive
Iron chloride hemotoxylin	Fibrin	Gray to dark blue	Positive
Gram-Weigert fibrin stain	Fibrin	Deep blue-black	Positive
Silver nitrate	Calcium	Orange black	Negative
Carbolfuchsin (Krajian)	Mucin	Magenta	Negative

Simple hyalin *(2)* is a substance, protein in nature, whose origin and specific composition are rather obscure. It resembles amyloid in its morphological characteristics but does not give the micro-chemical reactions of amyloid or mucin. The presence of hyalin generally indicates degeneration or cell death as deposits are frequently found in areas where the cells of the affected tissue lose their structure, the cytoplasm coagulates, and fusion takes place; however, hyalin deposits may be the secretion of epithelial cells around the nuclei of dead cells or other debris *(2, 8)*. It has been mentioned that fibrin, blood platelets, and leucocytes may undergo hyalin degeneration *(1, 5)*. Spencer and McNutt *(12)* found udders showing inflammatory points characterized by occlusion of the alveoli and ducts with fibrin and leucocytes. The observations made in this study suggest that the bodies were composed of leucocytes, and possibly other cellular debris, around which blood fibrin had been adsorbed and had undergone hyalinization.

Site of formation. Because the majority of the bodies were found lying free in the alveoli, McFaydean *(9)* concluded that the lumen of the alveolus was the site of formation. Joest and Steck *(7)*, however, considered the bodies to have their formation in the epithelial layer of the alveolus. In this study the bodies were usually found lying free in the alveoli and ducts with the epithelium intact. They were occasionally found in the interstitial tissue. It is probable that these bodies were first formed in the alveoli and later appeared in other areas as a result of necrosis of the epithelium or were translocated by the procedures of preparing and cutting the tissue. If the bodies were formed in the interstitial tissue, one would expect to find a larger number present in that location. Also, it is difficult to theorize their migration into the alveoli without leaving some evidence of the phenomenon.

Frequency of occurrence. Hyalin-fibrin bodies were observed in 13 of 22 lactating udders and in two dry udders. They were not found in the udder of the only virgin heifer studied. Microscopic examinations failed to reveal any positive association between the presence of these bodies and the proliferation of connective tissue, cellular infiltration of the alveoli or interstitial tissue, alteration of the epithelium, or glandular activity. Morrill *(10)* noted little or no correlation with age of the animal but thought the number present increased with advanced stage of lactation.

SUMMARY

Microscopic examinations revealed the presence of intra-alveolar bodies in 15 of 25 udders. The bodies were composed of a hyalin-fibrin complex lying free in the alveolus and ducts. They generally occurred singly; however, in some instances there were as many as three per alveolus. When an udder contained these bodies, they were found in approximately equal numbers in each quarter.

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HIGH- VS. LOW-PROTEIN GRAIN MIXTURES AS SUPPLEMENTS TO LEGUME-GRASS PASTURES

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Experiments were conducted to determine the minimum amount of supplemental protein that should be fed to milking cows grazing different pasture herbages. Grain mixtures containing approximately 10% protein were found adequate to support an average milk production of 30 lb. of 4% milk by Jersey cows on good pasture. *Editor.*

Research on the percentage of protein in grain mixtures that should be fed to supplement pasture is limited. Henderson and Reaves *(5)* point out: "The amount of protein in the grain mixture for cows on pasture need not be high when the pasture is good. Hence a grain mixture consisting of home-grown grains and containing not more than 12 to 14 per cent protein can be fed with good results." Morrison *(7)* states: "Dairy cows on such pastures (high in protein) are often fed a concentrate or grain mixture containing much more protein than is necessary. This does not increase their production and is usually decidedly uneconomical." Petersen *(10)* states specifically: "Unless the pasture grass is mature in the latter part of the pasture season, no attention need be paid to the protein content of the concentrate supplement as green herbage furnishes an abundance of protein. If the pasture is mature and consists largely of grasses, then it is necessary to furnish a concentrate with at least 10 per cent of digestible protein."

None of the authorities quoted above cited data to document the viewpoints they expressed. Balance trials *(4)* of cows on fertilized and unfertilized bluegrass without supplement show that grass alone may provide two or more times the amount of protein required.

In 1928 Hayden and Perkins *(4)* described an experiment in which two groups of cows were on good pasture in a reversal type experiment. The group that received 20% total protein in their concentrate mixture produced no more than those that received 12% protein. The following year *(9)* in a continuation of the experiment and starting on more mature grass and with a deficit of rainfall they obtained only 2% higher production from the group fed 20% protein. The results *(8)* of the third season on this experiment confirm those of the second, namely, slightly higher production from high protein but at a monetary loss. Horwood and Putnam *(6)* report that three cows on a grain ration containing 9.26% digestible crude protein produced 1% more 4% milk in 1931 and 4% more in 1932 than a similar group with 16.71% digestible protein in their grain ration. They regarded the differences as not of statistical significance.

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Darnell and Shepardson *(1)* later reported an experiment consisting of three trials. One group received a ration containing low protein and the other a high-protein ration. The percentages of protein were not reported. Both rations contained the same ingredients but with the proportions changed so that one ration contained one-third more protein than the other. During the first and third trials the group fed the high-protein ration produced more, whereas during the second trial the low-protein group produced slightly more. On an average for the three trials the group on the high protein produced 0.93 lb. more milk per animal daily. This does not appear to be a significant difference.

Ellenberger *(3)* in a pamphlet for wartime emergency use states that any grain mixture supplying satisfactory total digestible nutrients will contain enough protein, 10 to 12 per cent being ample to supplement good grass.

In grazing experiments conducted at this station prior to 1951, grain concentrate mixtures have been fed with the same protein content as that fed in the winter season. This practice was followed to make sure that protein was not a limiting factor for milk production, although protein was apparently being fed in excess of actual needs. Experiments reported here were conducted in an effort to determine the minimum amount of supplemental protein that should be fed to milking cows grazing different pasture herbages.

EXPERIMENTAL PROCEDURE AND RESULTS

In 1951 ten Jersey cows were grazed continuously on 9 acres of brome-alfalfa-Ladino and 10 other Jerseys were grazed rotationally on six 1½-acre paddocks of similar pasture. All cows were fed individually grain ration No. 35, containing 15.7% total crude protein and composed of the following: 450 lb. ground yellow corn, 300 lb. ground oats, 100 lb. wheat bran, 150 lb. soybean oil meal (41%), 10 lb. steamed bone meal, and 10 lb. iodized salt.

In 1952 the fencing was rearranged to allow three replications of both the continuously and the rotationally grazed areas of 3 acres each. Four cows that had been selected on the basis of similar current and previous milk production, date of calving, size, and age were assigned to each 3-acre lot. One-half of each group was fed grain ration No. 35 (15.7% protein, previously described) and the other half was fed ration No. 59. Ration No. 59 contained 10.1% crude protein and was composed as follows: 500 lb. of corn and cob meal, 500 lb. of ground oats, and 10 lb. of iodized salt.

The grazing experiment was continued during 1953 but with a change in grain feeding. Five animals were grazed on each 3-acre area. Two cows in each group received no grain, one received grain ration No. 59 (10.1% protein), and two received grain ration No. 35 without bone meal (15.9% protein). In 1953 the group assignment of cows varied from 1952 in that the 30 cows were divided into three groups of six and three groups of four based on similarity of current and past milk production, stage of lactation, and age. Each group of six was assigned at random to the paired pastures so that there were three on each. The three groups of four animals were also assigned at random to make a total of five animals on each three acres.

TABLE 1

Pounds of grain consumed per cow per day during the experimental grazing periods

	1951	1952		1953		
	High-protein ration	Low-protein ration	High-protein ration	Low-protein ration	High-protein ration	Av.
						(yr.)
Rotational grazing	4.6	5.5	5.8	4.3	6.2	5.2
Continuous grazing	4.6	5.5	5.8	4.8	5.9	5.2
Average, rations		5.5	5.8	4.5	6.0	
Average, years	4.6	5.6		5.5		
L.S.D. rations means at 1% level		0.3		N.S.		
C.V. grazing		4.6		20.2		
C.V. rations		2.9		32.0		
Coef. animal variability		7.9		27.9		

A limit of 1 lb. of concentrate for each 5 lb. of fat-corrected milk was set as an ideal, but the cows voluntarily restricted grain consumption to less than this amount. In the spring when they first went out on pasture many cows stopped or nearly stopped eating grain but gradually resumed eating it. In operation, the above policy resulted in each cow being allowed as much grain as she would consume. The cows were fed grain in the manger at milking time, the refusal was weighed back, and individual records of consumption were kept.

In Table 1 are shown the amounts of grain consumed per cow per day during the seasons of 1951, 1952, and 1953, which were of 121, 125, and 123 grazing days, respectively. It will be noted that during 1951, when only one grain ration was fed, there was no difference in grain consumption by groups grazing continuously and those grazing rotationally. This observation held true also in 1952 on each of the rations, but the cows fed the high-protein ration consistently ate more than those on the low-protein ration. The high-protein ration appeared to be more palatable because of the soybean oil meal and because it contained no cobs. Over the 3-year period the average grain consumption was the same whether the cows grazed continuously or rotationally.

The milk production data are presented in Table 2. It will be noted that the average daily milk production declined during the period 1951-1953. The inten-

TABLE 2

Pounds of 4% milk per cow per day during the grazing seasons

	1951	1952		1953	
	High-protein ration	Low-protein ration	High-protein ration	Low-protein ration	High-protein ration
Rotational grazing	34.6	35.5	29.8	28.4	31.3
Continuous grazing	34.0	30.6	34.4	29.2	30.8
Av. for rations		33.0	32.1	28.8	31.1
Av. for years	34.3	32.6		30.3	
Coefficient of variability, grazing		6.5		17.3	
Coefficient of variability, rations		5.9		17.0	
Coefficient of animal variability		21.4		20.5	

sity of stocking and the proportion of 2-year-olds in the herd increased. There were 10 cows on 9 acres in 1951, 12 in 1952, and 15 in 1953.

In 1953, the group grazing continuously consumed 8,100 lb. of hay while in the barn for milking, and those on rotational grazing consumed 7,600 lb. These amounts represent daily averages of 4.4 and 4.1 lb., respectively, a difference of 0.3 lb.

The cows fed no grain ate the same amount of hay daily as those fed the grain of low-protein content. Those fed high-protein grain ate 4.6 lb. of hay daily as contrasted with 4.0 lb. for the other groups.

DISCUSSION

Most of the reported experiments dealing with low vs. high protein in supplements fed with pasture grass were conducted one or two decades ago. Experimental techniques have improved in the interim. Replication of groups and years would seem to be a safe technique.

Reference to Tables 1 and 2 shows that as the stocking rate per acre increased from 1951 to 1953 the pounds of grain consumed per day increased and the daily milk yield decreased. These changes appear to be the result of an attempt through management to secure more complete utilization of the pasture crop.

Whether the cows grazed continuously or rotationally did not seem to influence the amount of grain consumed.

The cows on high-protein grain mixture consumed more than those on low protein whether grazing rotationally or continuously, indicating greater palatability of the ration containing soybean oil meal and no corn cobs. The cows on the low-protein ration sorted out and left cobs in the manger.

The writers are unable to attach any importance to the reversed relationship between low and high protein in the two systems of pasture management in 1952.

The lowered average daily milk production in 1953 is partially due to an increased proportion of 2-year-old cows in the milking herd and also to increased stocking rate. The 2-year-olds were equally distributed between the two systems of grazing and the two levels of protein feeding. The 2% variation in milk production between the two systems of management suggests no reason for hesitation to combine the two for analysis of milk production on each level of protein feeding.

SUMMARY

Average daily feed consumption and milk production data are presented which indicate that grain mixtures containing approximately 10% protein are adequate to support an average milk production of 30 lb. of 4% milk by Jersey cows on good pasture. Grain rations with higher protein content did not result in higher milk production.

ACKNOWLEDGMENT

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AN APPRAISAL OF THE SPLIT-SAMPLE METHOD IN ARTIFICIAL BREEDING STUDIES[1]

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The relative efficiency of the split and alternate-week semen samples for artificial breeding field trials showed a gain of 43.5% for the former. However, there is an additional cost involved in the preparation of split samples. *Editor.*

In conducting artificial breeding field trials there are, for all practical purposes, two sample sizes that may be used. One is the split sample. A semen sample is split into two equal portions, and planned semen treatments are applied to the split samples. The other is termed, in this report, the "alternate-week" sample. The semen samples are treated according to plan, with successive samples receiving different treatments.

Owing to the fact that the variable observed is the nonreturn rate, the size of semen sample available (the number of cows serviced) will have an influence on the precision of the estimate in accordance with binomial theory. In this respect, then, the split sample is at a disadvantage in that the observed nonreturn rates can be expected to be more variable. Also, the split sample requires more labor in its preparation. For these two reasons, many studies have been carried out by using the alternate-week sample.

In conjunction with recent studies *(1)* data were obtained from split and from alternate-week samples. These offer an opportunity to examine the relative efficiency of the two methods.

EXPERIMENTAL PROCEDURE

Data were available from nine field trials conducted from March, 1952, through November, 1953, during which time both alternate-week and split samples were used. The split samples were primarily from bulls collected from on weekdays during the trial periods, and the alternate-week samples were from bulls collected from on weekends.

Nonreturn rates were determined on 89-day nonreturns to service. Only samples or split-sample pairs used in ten or more first or second services were included in the statistical analysis. Thus, if one split sample of a pair had less than ten services, both members of the pair were eliminated.

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To counterbalance possible inseminator differences, the following patterns were adopted.

Split-sample trials. Inseminators were divided into two equal groups based on the number of first services performed by the inseminator during the year previous to the start of the field trials—that is, divided into two groups on the basis of experience. To balance the possible effect of inseminator groups, the samples were handled as follows:

	Week	Treatment	Inseminator group
Bull A	1	1	I
		2	II
	2	1	II
		2	I

Alternate-week trials. All inseminators received the same sample (treatment) in a given week. The paired treatments were applied to alternate samples with a random start. Schematically, samples from a bull followed a pattern of the following type:

	Week	Treatment	Inseminator groups
Bull B	1	1 or 2	All
	2	2 or 1	All

The data used in the analyses were the unweighted percentage nonreturn rates. Owing to the prevalence of unequal frequencies, all estimates of residual variation were obtained from application of least squares procedures.

RESULTS AND DISCUSSION

Table 1 summarizes the results of the statistical analyses for both the split sample and alternate-week trials. Since the two trials were running concurrently, with the same group of bulls, it is considered justifiable to calculate

TABLE 1

Analyses of variance from split sample and from alternate-week sample applications to semen treatment experiments

Source of variation	d.f.	S.S.	M.S.
Split samples			
Between bulls	21	26,036.58	
Samples within bulls	738	129,211.93	175.08
Treatments (adj. for bulls)	9	3,193.18	
Split-sample residual	751	54,179.65	72.14
Alternate-week samples			
Bulls and mean	23	2,867,939.15	
Treatments (adj. for bulls)	15	7,062.70	
Alternate week residual	540	55,916.65	103.55

the relative efficiency of the split-sample error to that derived from alternate-week samples. The relative efficiency was observed to be

$$\frac{103.55}{72.14} \times 100 = 143.5\%$$

Thus, there was a gain of 43.5% through use of split samples rather than alternate-week samples, indicating that the split sample in semen treatment experiments is an effective means of reducing residual variation. For a discussion on relative efficiency, the reader is referred to Ostle *(2)*.

This result is in line with what might be predicted from a knowledge of split-sample (plot) applications in other areas of research. The split-sample residual is free of seasonal influences as affecting fertility rates, as well as of sample-to-sample variations in nonreturn rates, which are known to be large.

Since the gain in efficiency was achieved at some additional expense entailed in the preparation of split samples, it is not an unencumbered gain. This additional cost would have to be given due consideration when considering the relative merits of the two types of samples.

SUMMARY

A comparison of residual variation derived from split samples and from alternate-week samples indicates a gain in efficiency of 43.5% through use of split samples in semen treatment experiments.

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ESTEROLYTIC ACTIVITY OF CERTAIN ALIMENTARY AND RELATED TISSUES FROM CATTLE IN DIFFERENT AGE GROUPS[1]

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This investigation had as its objectives additional information relative to the location of the tissues from which pregastric esterase originates in the bovine and to study the effect of age of the animal upon this enzyme system. Extracts from animals of three age groups were prepared (8 to 36 hours, 2 to 3 months, and 1 to 3 years). The four areas from which pregastric esterase is most likely to originate were found to be the vallate papillae region of the tongue, the glosso-epiglottic area, the pharyngeal end of the esophagus, and the submaxillary salivary gland. *Editor.*

Food undergoes numerous chemical alterations during its passage through the mammalian alimentary tract. The physiological importance of many of these changes occurring in the gastric and postgastric areas has been established. Little significance, however, has been accorded those processes to which food is subjected in the pregastric area. Saliva, which is regarded as the primary glandular secretion entering the pregastric portion of the alimentary tract, has been reported to contain several digestive enzymes. Among these salivary enzymes is esterase,[3] which has been observed in humans *(8, 10, 11, 17)*, sheep, oxen *(10)*, and dogs *(8, 11, 16)* but has not been demonstrated to be of physiological importance.

An esterase that originates anterior to the medial portion of the esophagus in the dairy calf has been detected *(23)* and subsequently studied *(24, 25)* by observing changes that occurred in various milk products after being "sham-fed" to either rumen- or esophageal-fistulated calves. The physiological significance of this enzyme system is undetermined. The specific location and the identity of the glands that secrete pregastric esterase in the calf also are unknown, although the preparation of an esterase-active extract, a "cheese modifying enzyme product," from certain tissues in the pharyngeal region of the calf

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[2] The data in this paper are from a thesis submitted by the senior author, a Ralston Purina Research Fellow, to the graduate faculty of North Carolina State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] In this manuscript, the term "esterase" is employed in a general sense to mean any enzyme capable of catalyzing the following reaction:

$$R \cdot COOR + H_2O \leftrightarrow R \cdot COOH + HO \cdot R$$

TABLE 1

Tissues sampled for esterase assays

Sources of tissue	Description of sample
Oral cavity	
Tongue, region of the fungiform papillae	A section, approximately ¼ in. thick, of the dorsum of the tongue immediately anterior to its prominence (Area A in Figure 1).
Tongue, region of the vallate papillae	A section, approximately ¼ in. thick, of the dorsum of the tongue in the region of the vallate papillae (Area B in Figure 1).
Glosso-epiglottic area	A section of the dorsum at the root of the tongue, the lining of the glosso-epiglottic space, the oral surface of the epiglottis and a portion of the soft palate (Area C in Figure 1).
Salivary glands	
Parotid Sublingual Submaxillary	A random sample of each of the different salivary glands.
Esophagus	
Pharyngeal end	The lining of the anterior end, approximately 1.5 in., of the esophagus (Area D in Figure 1).
Anterior portion	The lining of the anterior half of the esophagus, exclusive of the part defined as the pharyngeal end.
Posterior portion	The lining of the posterior half of the esophagus, exclusive of the part defined as the pharyngeal end.
Abomasum	
Pyloric region	A section of the lining (in cattle) or the entire wall (in calves) of the pyloric region of the abomasum.
Fundic region	A section of the lining (in cattle) or the entire wall (in calves) of the fundic region of the absomasum.

has been reported *(6)*. Thus, the investigation presented herein was initiated to gain additional information relative to the location of the tissues from which pregastric esterase originates in the bovine and to study the effect of age of animal on the activity of this enzyme system.

EXPERIMENTAL PROCEDURE

Extracts of various alimentary and related tissues from animals of three age groups (8 to 36 hours, 2 to 3 months, and 1 to 3 years) were prepared and assayed for their esterolytic activity.

Source of tissue. The various tissues investigated are identified in Table 1. The choice of tissues in the pregastric area was based on recognized general physiological functions and on the observed esterolytic activity of tissues from the pharyngeal region *(6)*. Sections of the abomasum were included for comparative purposes. The anatomical locations of some of the tissues removed from the pregastric area are shown diagrammatically in Figure 1. Tissues were removed immediately after the animals were slaughtered. Samples of all the tissues listed in Table 1, however, were not taken from every test subject.

Preparation of tissue extracts. Samples of fresh tissue (5 to 15 g.) were minced with scissors and subsequently homogenized with 10% glycerol, in the

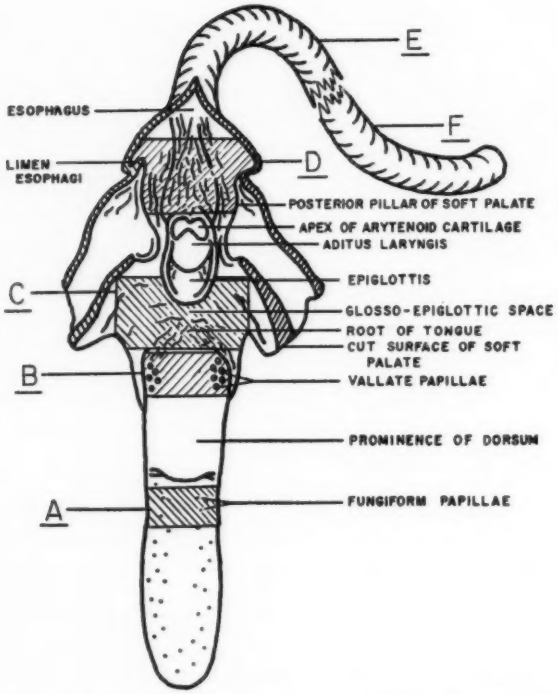

FIG. 1. Dorsal view of the tongue, pharynx, and esophagus of the bovine in which the soft palate, pharynx, and origin of the esophagus are cut dorsally and reflected (adapted from Sisson and Grossman *(19)*, pp. 450 and 549). A = tongue, region of the fungiform papillae. B = tongue, region of the vallate papillae. C = glosso-epiglottic area. D = esophagus pharyngeal end. E = esophagus, anterior portion. F = esophagus, posterior portion.

ratio of 1 g. of fresh tissue to 10 ml. of 10% glycerol, in a Waring blendor for 2 minutes. The homogenate was filtered, and a portion of the filtrate, which will be referred to subsequently as the "original tissue extract," was collected for assay. Unless indicated otherwise, the original tissue extracts were assayed without dilution. Assays were conducted as soon as possible, not longer than 2 to 3 hours, after preparation of the tissue extracts.

Esterase assay. The assay technique employed in this investigation was identical in principle to that described by Nachlas and Seligman *(15)*, but some of the reagents and parts of the procedure were changed.

A. Reagents:

1. *Ester-dioxane solutions* were 5.23×10^{-3} *M* solutions of 2-naphthyl laurate[4] (m.p. 59.5-61.0° C.) and of 2-naphthyl palmitate[4] (m.p. 69.0-70.5° C.) in re-

[4] Synthesized in this laboratory by the senior author.

distilled 1,4-dioxane. These solutions were refrigerated when not in use. Dioxane was selected as a carrier for the 2-naphthyl esters because preliminary observations indicated that this solvent was less inhibitory toward enzymatic hydrolysis than was acetone, which was used by Nachlas and Seligman *(15)*. Equimolar concentrations of substrate were used to enhance accuracy of comparison of the relative specificities of tissue extracts for each of the 2-naphthyl esters.

2. *Naphthanil Diazo Blue B.* A commercial preparation of tetrazotized 4,4′-diamino-3,3′-dimethoxybiphenyl.[5]

3. *Sodium taurocholate*, pure.

4. *M/15 phosphate buffers.* These were prepared by mixing together various proportions of *M*/15 solutions of KH_2PO_4 and Na_2HPO_4 to obtain the desired pH (6.6, 7.2, or 7.8), which was checked during preparation by means of a pH meter. Nachlas and Seligman *(15)* used a veronal buffer, but they stated subsequently that when this reagent was stored either at 4° C. for longer than a month or at room temperature, its use resulted in nonenzymatic hydrolysis of the 2-naphthyl esters *(18)*.

5. *40% trichloroacetic acid.*

6. *Ethyl acetate.*

The buffered substrate mixture was prepared by swirling the following components in a beaker: 40 ml. of buffer, 55 ml. of distilled water, and 75 mg. of sodium taurocholate. To this swirling mixture, 5 ml. of a dioxane-ester solution were added from a pipette, the tip of which was submerged in the mixture.

B. *Measurement of esterolytic activity.* Five ml. of a buffered substrate mixture and 1 ml. of tissue extract were mixed in a test tube and incubated in a water bath at 37° C. for a specified time (laurate, 32 minutes; palmitate, 2.5 hours). At the end of the incubation period, 1 ml. of a chilled aqueous solution, freshly prepared, containing 5 mg. of Naphthanil Diazo Blue B was added to the enzyme-substrate mixture. Four minutes later, 1 ml. of 40% trichloroacetic acid was added. Ten ml. of ethyl acetate then were added, and the tube was shaken vigorously. The tube was centrifuged to separate the ethyl acetate layer, which contained the azo dye, from the aqueous layer. The optical density of the ethyl acetate layer was measured at a wave length of 540 mμ in a Beckman DU spectrophotometer. The reagent blank was the ethyl acetate layer of an incubated control in which the tissue extract had been heated to inactivate the esterase. Optical density was converted to per cent hydrolysis by means of a calibration curve.

Initially, assays were conducted at pH 7.2 and pH 7.8. Subsequently, pH 6.6 also was adopted because extracts from certain tissues exhibited maximum activity at this point. Both the laurate ester and the palmitate ester were employed as substrates to gain preliminary information on the relative substrate specificity of various tissue extracts. Inasmuch as all of the tissue extracts hydrolyzed the palmitate ester more slowly than they did the laurate ester, the length

[5] Manufactured by E. I. du Pont de Nemours and Company, Wilmington, Del.

TABLE 2

Hydrolysis of 2-naphthyl laurate at pH 6.6 by extracts[a] of various alimentary and related tissues

Sources of tissue	Hydrolysis of substrate by age groups	
	Calves (8-36 hours)	Cattle (1-3 years)
Oral cavity	*(%)*	*(%)*
Tongue, region of the fungiform papillae	0.2 ± 0.3[b] (4)[c]	0.1 ± 0.1[b] (6)[c]
Tongue, region of the vallate papillae	14.5 ± 5.2 (4)	0.9 ± 0.5 (6)
Glosso-epiglottic area	29.5 ± 13.0 (4)	3.2 ± 2.4 (6)
Salivary glands		
Parotid	1.1 ± 0.1 (4)	0.5 ± 0.2 (6)
Sublingual	1.0 ± 0.3 (4)	2.0 ± 0.8 (6)
Submaxillary	3.8 ± 1.5 (4)	5.3 ± 1.8 (6)
Esophagus		
Pharyngeal end	30.7 ± 15.7 (4)	1.4 ± 0.3 (3)
Abomasum		
Pyloric region	3.5 ± 3.8 (3)	4.6 ± 0.8 (3)
Fundic region	4.1 ± 2.4 (3)	2.2 ± 0.5 (3)

[a] The original tissue extracts were diluted (1:5) with 10% glycerol prior to assay.

[b] Mean ± standard error.

[c] Total number of observations on which each mean is based.

of incubation was increased whenever the former was used as the substrate. Although these different factors in assay procedure were varied from time to time, sufficient uniformity was maintained to permit valid comparisons later.

RESULTS

Results from the esterase assays of extracts of the various tissues are summarized according to substrate and pH in Tables 2, 3, 4, and 5. In general, extracts of tissues from the tongue in the region of the vallate papillae, from the glosso-epiglottic area and from the pharyngeal end of the esophagus exhibited more esterolytic activity than did extracts of tissues from the tongue in the region of the fungiform papillae (Tables 2, 3, 4, and 5) and from the anterior and the posterior portions of the esophagus (Table 3). Extracts of the first three aforementioned tissues from the youngest animals, however, hydrolyzed the substrate decidedly more rapidly than did extracts of corresponding tissues from the older animals. These differences in the rates of hydrolysis became more pronounced as the pH was reduced from 7.8 to 6.6.

TABLE 3

Hydrolysis of 2-naphthyl laurate at pH 7.2 by extracts of various alimentary and related tissues

Sources of tissue	Hydrolysis of substrate by age group
	Calves (2-3 months)
	(%)
Oral cavity	
Tongue, region of the fungiform papillae	1.0 ± 0.6[a] (4)[b]
Tongue, region of the vallate papillae	7.1 ± 1.5 (4)
Glosso-epiglottic area	11.4 ± 4.3 (4)
Esophagus	
Pharyngeal end	10.8 ± 7.7 (4)
Anterior portion	1.1 ± 0.3 (4)
Posterior portion	1.1 ± 0.8 (4)

[a] Mean ± standard error.
[b] Total number of observations on which each mean is based.

Differences in the rates of hydrolysis by extracts of the various salivary glands were apparent only when 2-naphthyl laurate was employed as the substrate (Tables 2 and 4). The esterolytic activity of the submaxillary was highest; that of the sublingual usually was intermediate, and that of the parotid nearly always was lowest. Differences in the activity of salivary gland extracts from animals of different age groups were much less pronounced than were observed for extracts of tissues from the oral cavity and the esophagus. A slight increase in activity with increasing age was noted for the submaxillary and the sublingual glands. This is in contrast to observations on extracts of tissue from the tongue in the region of the vallate papillae, from the glosso-epiglottic area and from the pharyngeal region of the esophagus, in which tissues the activity decreased markedly with increasing age. Furthermore, within the pH range studied (6.6 to 7.8), the activities of salivary gland extracts were affected only slightly by changes in hydrogen ion concentration.

The rate of hydrolysis effected by extracts of the fundic and the pyloric regions of the abomasum were similar (Tables 2, 4, and 5). No marked differences due to age of animal were apparent. The rate of activity for both tissue areas was enhanced somewhat by reducing the pH from 7.8 to 6.6 (Tables 2 and 4). None of the abomasal extracts, regardless of age of animal, were as high in activity as were extracts of the following tissues from day-old calves: the tongue in the region of the vallate papillae, the glosso-epiglottic area, and the pharyngeal end of the esophagus.

DISCUSSION

The degree of esterolytic activity detected in the various tissues indicates that the four areas from which pregastric esterase appears most likely to originate

TABLE 4

Hydrolysis of 2-naphthyl laurate at pH 7.8 by extracts of various alimentary and related tissues

Sources of tissue	Hydrolysis of substrate by age groups		
	Calves		Cattle
	(8-36 hours)	(2-3 months)	(1-3 years)
	(%)	*(%)*	*(%)*
Oral cavity			
Tongue, region of the fungiform papillae	0.8 ± 0.4[a] (5)[b]	0.2 ± 0.2[a] (7)[b]	0.8 ± 0.7[a] (6)[b]
Tongue, region of the vallate papillae	4.5 ± 2.1 (5)	2.9 ± 2.3 (7)	1.4 ± 0.2 (6)
Glosso-epiglottic area	13.4 ± 3.9 (5)	2.0 ± 0.9 (7)	2.9 ± 1.2 (6)
Salivary glands			
Parotid	3.4 ± 0.8 (5)	1.7 ± 0.2 (4)	3.8 ± 1.6 (6)
Sublingual	3.3 ± 1.0 (5)	8.9 ± 11.9 (4)	8.8 ± 4.1 (6)
Submaxillary	15.2 ± 3.4 (5)	18.3 ± 2.9 (7)	22.6 ± 2.8 (6)
Esophagus			
Pharyngeal end	16.6 ± 12.6 (5)	2.1 ± 1.4 (7)	3.3 ± 0.3 (3)
Abomasum			
Pyloric region	3.3 ± 2.0 (4)	———	5.8 ± 2.2 (3)
Fundic region	2.9 ± 0.8 (4)	———	2.4 ± 0.2 (3)

[a] Mean ± standard error.
[b] Total number of observations on which each mean is based.

are the vallate papillae region of the tongue, the glosso-epiglottic area, the pharyngeal end of the esophagus, and the submaxillary salivary gland. Two of these, the glosso-epiglottic area and the pharyngeal end of the esophagus, are among the tissues of the pharyngeal region utilized by Farnham *(6)* in the preparation of an esterase-active "cheese modifying enzyme product."

Although it was assumed that extracts of pregastric tissues that secrete esterase probably would be higher in esterolytic activity than those of pregastric tissues that do not secrete this enzyme, it was realized that esterases have been detected also in nonsecretory cells of a large variety of animal tissues. Thus, extracts of the foregoing pregastric tissues might have contained esterase from either secretory or nonsecretory cells or from both. The observation that such extracts were high in esterolytic activity, therefore, can be accepted only as implicatory evidence concerning the source of pregastric esterase.

The prevalent concept of the cellular origin of enzymes present in the secretions of exocrine glands is that such enzymes are produced by the serous cells and not the mucous cells *(5)*. Thus, it is of interest to consider the various pregastric tissues sampled in this study in relation to the cellular nature of glands

TABLE 5

Hydrolysis of 2-naphthyl palmitate at pH 6.6 by extracts of various alimentary and related tissues

Sources of tissue	Hydrolysis of substrate by age groups	
	Calves (8-36 hours)	Cattle (1-3 years)
	(%)	*(%)*
Oral cavity		
Tongue, region of the fungiform papillae	0.4 ± 0.3[a] (2)[b]	0.2 ± 0.3[a] (3)[b]
Tongue, region of the vallate papillae	7.0 ± 1.1 (2)	2.1 ± 0.2 (3)
Glosso-epiglottic area	11.8 ± 0.9 (2)	4.1 ± 2.0 (3)
Salivary glands		
Parotid	2.8 ± 1.6 (2)	3.4 ± 0.3 (3)
Sublingual	2.1 ± 0.5 (2)	1.9 ± 0.4 (3)
Submaxillary	2.3 ± 1.3 (2)	2.1 ± 0.2 (3)
Esophagus		
Pharyngeal end	22.4 ± 7.6 (2)	———
Abomasum		
Pyloric region	3.7 ± 0.0 (2)	6.9 ± 0.9 (3)
Fundic region	5.3 ± 0.8 (2)	6.7 ± 1.7 (3)

[a] Mean ± standard error.

[b] Total number of observations on which each mean is based.

known to be present therein and the esterolytic activity of extracts prepared therefrom. The glands of Ebner, which are located beneath the vallate papillae, are entirely serous *(20)*. Mixed glands, those containing both serous and mucous elements, are found at the root of the tongue. Extracts of tissue from calves in both of these areas were high in activity. The esophageal glands, which are found only at the pharyngoesophageal junction in the bovine, are reported to be mucous in character and thus would not be expected to manifest enzymatic activity. To the contrary, extracts of the pharyngeal end of the esophagus in calves were high in esterolytic activity. Extracts of the submaxillary salivary gland, which contains both serous and mucous cells, showed more activity than those of the parotid and the sublingual salivary glands, which are serous and mixed glands, respectively. Extracts of the cardial end of the esophagus, which area is glandless, and of the anterior portion of the tongue, in which only mucous glands are found, exhibited very little esterolytic activity.

In three pregastric tissues (the tongue in the region of the vallate papillae, the glosso-epiglottic area, and the pharyngeal end of the esophagus) from the

day-old calf, the esterolytic activities were high but diminished markedly with increasing age of animals. This downward trend in the in vitro activity of tissue extracts suggests that the secretion of pregastric esterase in vivo also may undergo a corresponding diminution with increasing age. In view of the possible physiological importance of pregastric esterase, this suggested decrease in its secretion rate merits consideration.

In the early life of some mammals, the concentrations of lipase in gastric and postgastric secretions are lower than those that are typical in adulthood. In newborn infants that are premature, both the gastric contents *(14)* and the duodenal juice *(3)* have been reported to contain less lipase than those of infants that are born at term. Andersen and Dueholm *(1)* noted that duodenal juice obtained from 0- to 3-month-old infants possessed only one-third as much lipolytic activity as that obtained from 1- to 2-year-old infants. Additional observations also have indicated that the lipolytic activity of duodenal juice from infants increases with advancing age but remains lower throughout infancy and childhood than that which is characteristic of adulthood *(2, 3, 4, 9)*. Kryuchkova *(13)* reported that the pancreatic juice of dogs during the first 12 days of age contained no lipase. At 12 to 14 days, a slight increase in the activity of this enzyme was noted, and at 1.5 months of age, the activity rose to a level typical of adult dogs. Inasmuch as the gastro-intestinal secretion of lipase has been reported to be low in the early life of both humans and dogs, it is possible that a similar phenomenon may occur also in cattle, thereby increasing the relative importance of pregastric esterase.

In support of this possibility is the observation by Koref and Mũnoz *(12)* indicating that the concentration of lipase in fresh pancreatic tissue from calf embryos, suckling calves, and mature cows, respectively, increases in the order listed. In addition, the secretion of gastric lipase *(7, 22)* and of intestinal lipase *(21)* in cattle is believed to be almost nonexistent. In the study reported herein, extracts of gastric tissue from day-old calves exhibited considerably less esterolytic activity than did those of several pregastric tissues. Thus, in view of the aforementioned observations, it is conceivable that the young calf may rely on its secretion of pregastric esterase to a considerable extent for fat digestion.

SUMMARY

Extracts of various alimentary and related tissues from cattle of different ages were prepared and assayed for their esterolytic activity. Extracts of tissues from the tongue in the region of the vallate papillae, from the glosso-epiglottic area, from the pharyngeal end of the esophagus, and from the submaxillary salivary gland were higher in activity than extracts of tissues from the tongue in the region of the fungiform papillae, from the sublingual salivary gland, from the parotid salivary gland, from the portion of the esophagus distal to the pharyngeal end, and from the fundic and the pyloric regions of the abomasum.

Extracts of the tissues from the tongue in the region of the vallate papillae, from the glosso-epiglottic area, and from the pharyngeal end of the esophagus of day-old calves were more active than extracts of corresponding tissues from

older calves and mature cattle. The activity of extracts of the submaxillary and the sublingual salivary glands increased slightly with increasing age of animal.

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THE EFFECT OF DIFFERENT LEVELS OF IODIDE FEEDING ON SERUM INORGANIC AND PROTEIN-BOUND IODINE, WITH A NOTE ON THE FREQUENCY OF ADMINISTRATION REQUIRED TO MAINTAIN A HIGH LEVEL OF SERUM INORGANIC IODINE[1]

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This study was designed to determine the effects of normal dietary intake of supplemental iodine (KI) on the serum inorganic iodine and the protein-bound iodine levels of cattle. The application of the results to the frequency of iodine treatment in actinomycosis and other mycotic infections is made. Two mature cows were selected for the experiment. *Editor.*

Previous experiments *(3)* showed that the feeding of thyroprotein at the rate of 15 or 25 g. per day resulted in high levels of serum inorganic iodine. The protein-bound iodine (PBI) levels in the serum also were much higher than would be expected in the absence of any clinical evidence of hyperthyroid activity. These high PBI levels were assumed to be partly due to the formation of noncalorigenic PBI resulting from the combination of inorganic iodine in thyroprotein with certain serum protein constituents *(3)* and also to the presence of noncalorigenic iodinated compounds other than thyroxine in the thyroprotein, which have been demonstrated by Friedberg and Reineke *(2)*.

The study reported here was designed to determine the effects of normal dietary intake of supplemental iodine and the feeding of increasing amounts of KI above normal supplemental levels on the serum inorganic iodine and PBI levels of cattle. In this way the effects of dietary inorganic iodine on PBI, uncomplicated by oral intake of protein-bound iodine, could be determined.

Since, in iodine therapy for actinomycosis and other mycotic infections in cattle there is a wide variation in the recommended frequency of dosing, it was of special interest in this experiment to observe the length of time that high blood iodine levels were maintained after discontinuance of iodine feeding. Milks *(5)* recommended 15-30 g. of sodium iodide in 500 cc. distilled water intravenously, repeating every 4-5 days until iodism is produced. Merchant *(4)* recommended 30 g. of sodium iodide in 500 cc. distilled water administered intravenously at 18-day intervals.

The results will be discussed in relation to their possible application to the frequency of iodine treatment in actinomycosis and other mycotic infections.

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EXPERIMENTAL PROCEDURE

Two mature Jersey cows weighing 900-1,000 lb. were selected for the experiment. Iodized salt was withdrawn from the ration of each for a period of several weeks so as to insure basal serum iodine levels. During the first week of the experimental period 11 mg. of potassium iodide were added to the ration of each animal. This quantity was calculated to be the approximate amount of supplemental iodide fed daily in 12 lb. of grain containing 1% iodized salt (0.02% KI). This quantity was fed daily for 1 week. The quantity of KI fed daily was doubled each week for three successive weeks. Beginning with the fifth week the quantity was increased by 50% each week for the remaining 10 weeks, so that during the last week 2.816 g. of KI were being fed daily. During the entire KI feeding period blood samples were taken in the morning prior to feeding KI, or about 24 hours after the previous feeding. This was done to avoid the transient rise in serum iodine expected if the blood sampling was done shortly after the oral administration. Blood analyses for inorganic iodine and protein-bound iodine were made at the end of each weekly period by the method of Connor *et al. (1)*. When the higher levels of iodine were fed, extra washing of the precipitated protein was employed to minimize contamination of the PBI fraction by inorganic iodine. After 14 weeks of iodine feeding, all supplemental iodine was withdrawn from the ration of both cows. Blood samples were drawn daily for a time, followed by weekly sampling, in order to follow closely the pattern of decline of both inorganic iodine and PBI as they returned to normal levels.

RESULTS AND DISCUSSION

Relation of the amount of supplemental KI fed to the serum inorganic iodine level. The supplementary feeding of KI at levels of 11 mg. and 22 mg. per day

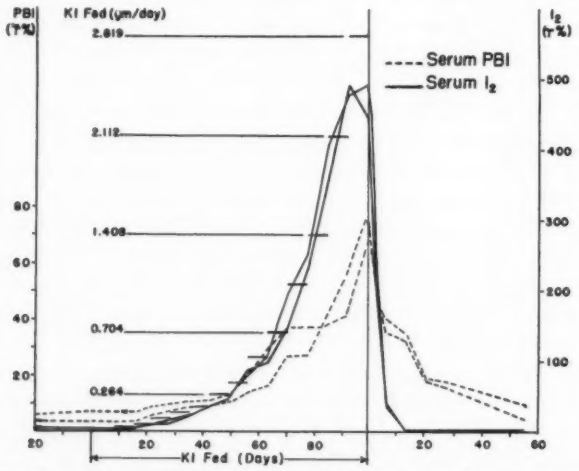

FIG. 1. The effect of increasing oral doses of KI on the blood serum level of PBI and inorganic iodine.

resulted in no effect on the serum inorganic iodine level (Figure 1). It is reasonable to assume that there may be a mechanism of homeostasis in operation which tends to keep the serum iodine level constant at these levels of iodine intake. When the KI feeding level was increased to 44 mg. or more per day, the quantity of inorganic iodine in the serum rose approximately in direct proportion to the quantity fed (Figure 1). This change occurred in both cows. When the level of 2.816 g. of KI per day was reached, additional KI failed to increase the inorganic iodine in the serum proportionately as much as would be predicted from results of previous lower dosages.

After the termination of the KI feeding, blood samples were drawn at 24-hour intervals for the first 3 days and again at the end of each week for 56 days (Figure 1). The serum inorganic iodine level fell precipitously. At the end of 24 hours after the last regular feeding period the serum inorganic iodine level dropped to about 85% of the highest levels; at 48 hours to 69%; at 96 hours to 46%; at 1 week to 16%; and at 2 weeks to 0.8%, which was the control level.

Thus, it appears that where iodism is being induced to treat actinomycosis and other mycotic infections, oral KI feeding should be administered no less often than at 2-day intervals in order to maintain a high level of blood inorganic iodine. Dosage intervals greater than 2 days accordingly would not be indicated where it is desired to maintain the serum at a continuously high level.

Relation of the amount of supplemental KI fed to the serum protein-bound iodine level. With the feeding of potassium iodide at levels as high as 22 mg. per day there was no effect on the serum protein-bound iodine (PBI) level. This observation was considered to be significant since it showed that the PBI test was not affected by the supplemental iodine intake likely to be encountered under usual conditions of feeding. With levels of 44 mg. or above of KI per day, the quantity of PBI in the serum rose roughly in proportion to the amount fed, up to 792 mg. per day. After this quantity was exceeded, the PBI level remained high but varied considerably.

It is thus reasonable to assume that with supplemental levels of iodine equivalent to 44 mg. and above of KI per day noncalorigenic iodine-containing compounds were formed which interfered with the use of the PBI test as a measure of thyroid status.

The results of this experiment show that as long as the inorganic iodine level is within normal limits, iodine intake can be assumed to be within limits that will not interfere with the PBI test of thyroid activity. The policy of using the inorganic iodine level as a guide in determining whether or not a blood sample is iodinated (in case of incomplete history) is thus well founded.

No evidence of toxicity from iodism or hyperthyroidism was noted at any of the levels of iodine feeding.

Unpublished results of in vitro experiments indicate that cellulose digestion by rumen organisms was not impaired by the presence of an amount of iodine occasioned by feeding as much as 2.8 g. of KI per day.

SUMMARY AND CONCLUSIONS

An experiment was conducted in which increasing amounts of potassium iodide were fed to two adult Jersey cows over a 14-week period. The first amount fed was 11 mg. (8.4 mg. I_2) per day, an amount approximately equal to half the recommended daily allowance of supplemental iodine in iodized salt. This level was maintained for a week and then increased at weekly intervals until finally 2.816 g. KI (2.15 g. I_2) per day were being fed. Blood samples were drawn for serum inorganic iodine and protein-bound iodine analysis at the end of each feeding level and at frequent intervals after the termination of KI feeding.

Levels of supplemental iodine intake as high as 22 mg. of KI per day had no influence on the serum inorganic iodine level. This suggested that a mechanism of homeostasis was in operation which served to keep this level constant. At 44 mg. of KI per day, the serum inorganic iodine level began to rise and continued to rise in direct proportion to the quantity fed, up to 2.816 g. per day. From a practical standpoint, the serum inorganic iodine up to the level of 500% can be effected by merely adjusting the amount of iodine fed daily within these limits of KI intake. After the termination of KI feeding, the serum inorganic iodine level fell 50% during the first 3 days and to the basal level by the end of 2 weeks.

In order to maintain a constantly high therapeutic level in the systemic treatment of mycotic infections, no more than 2 days should elapse between treatments. This would serve to keep the serum inorganic iodine level between 75 and 100% of the highest experimental level.

It was found that a supplemental KI intake as high as 22 mg. per day had no effect on the PBI level. This quantity is about twice that likely to be consumed in the recommended daily supplemental feeding of iodine in iodized salt. The PBI test therefore would not be affected by the usual level of supplementary iodine intake.

No evidence of toxicity from iodism or hyperthroidism was noted at any of the levels of iodine feeding.

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TECHNICAL NOTE

A SUGGESTED MODIFICATION OF THE TOLUENE DISTILLATION METHOD FOR MOISTURE DETERMINATION IN MILK POWDERS

In the toluene distillation method for determining moisture in milk powders, as specified by the American Dry Milk Institute, several difficulties may be encountered.

a) Toluene is inflammable and presents a hazard unless the cork stoppers fit perfectly.
b) Heaters are usually of the open coil element type and add to the fire hazard.
c) Heaters with rheostat type controls and open coil elements are difficult to adjust to maintain a constant distillation rate.
d) The flat bottom of the Erlenmeyer flask, with local exposure to the high heat, gives conditions ideal for scorching the powder sample unless the determination is watched almost constantly and the apparatus shaken frequently.

These difficulties may be overcome by certain modifications in the equipment.

In the suggested modification the following apparatus changes are made.

a) Standard taper, 24/40, ground joints are used for all connections.
b) The receiver is a Pyrex, Bidwell and Sterling type graduated to 5 ml. in 0.1-ml. divisions.
c) The 300-ml. Erlenmeyer flask is replaced by a 500-ml. round bottom, short neck boiling flask.
d) The heater with rheostat is replaced by a Glas-col heating mantle and a variable transformer is used to control the heat.

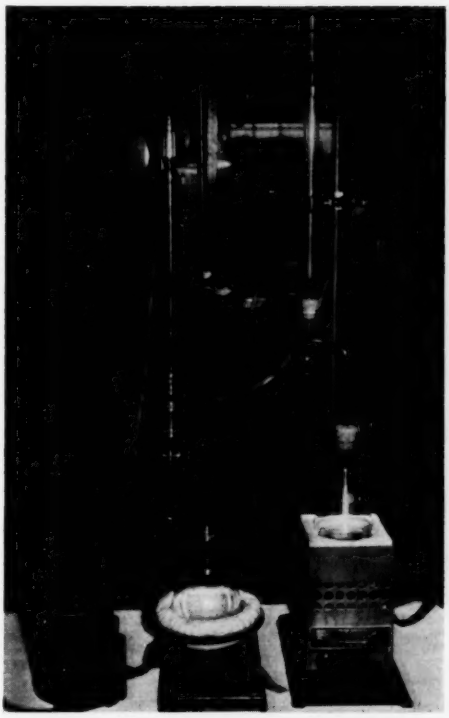

FIG. 1. Apparatus used for modified toluene distillation method for moisture determination in milk powder.

Discussion

This apparatus has been used extensively and has been found to give very good results. Fire hazard is eliminated by the ground joints and by the completely enclosed design of the heating mantle. Control of distillation rate is simplified owing to the partial shielding of the flask by the mantle, the fineness of control on the transformer, and the even distribution of heat. Absence of a local hot spot on the flask minimizes scorching. The round bottom of the flask permits more vigorous convection currents than does the flat bottom of an Erlenmeyer flask. This tends to keep the powder in suspension and helps further to reduce scorching. In addition, the set-up is not as tall as the apparatus now recommended. This makes brushing down and flushing the condenser more convenient.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Milk Flavor Uniformity Is Possible

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Feed and weed flavors have been a problem of considerable importance for many years throughout the dairy world. Until recently, attempts to overcome the problem in fluid milk have been aimed almost exclusively at farm production methods, whereas off-flavor removal equipment has been used in the butter industry for years.

Equipment, which is economical to use and which is acceptable to public health agencies, is now available for removing off-flavors and odors from fluid milk. By using this equipment, the dairy processor is able to offer his consumers a product which is more consistent in day-to-day flavor than was possible before.

Much of the remaining discussion will deal with the principles of feed flavor removal and typical removal equipment, its operation and control.

Feed Flavor Problem

To assure that the importance of the problem of feed and weed flavors is not minimized, a brief background is in order.

That the problem is world wide is amply shown by the literature. McDowall of New Zealand in *The Buttermakers Manual (9)* devotes a complete chapter to weeds and weed flavor, and another to their removal from cream. Hunziker, in his text, *The Butter Industry (8)*, likewise discusses the problem and states that vacuum treatment of cream for deodorizing has been practiced for many years. In 1954, Cuttell *(1)* in England described new deodorizing equipment for cream treatment in Europe. From 1935 to 1951 a number of patents for methods and apparatus for the deodorizing of dairy products were issued to H. L. Murray of New Zealand *(10-16)*. Other patents for methods and equipment for deodorization were issued to H. C. Horneman *(6, 7)* and to B. W. Hammer *et al. (4, 5)*. Wilster *(17)* at the Oregon Experiment Station found that an advantage in the use of the Vacreator for processing cream was the removal of feed and weed flavors. It was shown by Downs *et al. (2)* that more than 44% of the milk samples used in Collegiate Student Dairy Products Judging Contests since World War II contained defects listed as feed or weed, either alone or in combination with other flavor defects. Dunkley *(3)* reported that in a survey, off flavors caused by feeds were found in 127 out of 169 samples of pasteurized milk collected in eight widely separated cities. This is 75% of the samples!

No milk producing area is completely free of feed flavor and odor problems.

Off-Flavors Due to Feeds and Weeds Can Be Prevented

Dunkley *(3)*, McDowall *(9)*, and many others have pointed out ways to control feeding of herds and regulate milking times to prevent feed flavors from showing up in the milk.

Dunkley points out that recent trends in milk production have brought about changes in the flavor problems. A few years ago, when less attention was given to sanitation and few mechanical home refrigerators were available, defects caused by bacterial action limited the keeping quality of the milk. Today, with modern sanitary practices, both in the plant and on the farm, defects caused by bacterial action are rarely encountered in fluid milk, and milk is usable for a much extended period. This extra time sometimes permits off-flavors due to oxidation and rancidity to develop. The development of these off-flavors is also influenced by production methods.

Since there are fewer developed flavors to mask existing feed flavors, the feed flavors are more apparent. When the intensity changes or there are variations in the type of flavor, consumers become critical and consumption of milk decreases. A milk of natural flavor, consistently free of off-flavors and odors, is desired.

Dunkley said that flavor-producing substances in feeds are carried by the blood from the digestive system to the udder and appear in the milk. Some substances can be transmitted through the lungs to the blood and finally to the udder. These latter substances may appear in the milk as soon as 1 minute after the cow is fed. Most of the flavors transmitted from the digestive tract require about 20 minutes before becoming detectable in the milk. The flavor intensity increases for about 2 hours, then decreases. Ordinary feeds cause no flavor in the

milk if fed after milking and if withheld 4 to 5 hours before milking.

Occasional appearance of flavors from some feeds ordinarily not troublesome is an inconsistency making the prevention problem difficult. Sugar beets, beet pulp, and beet tops fall into this category.

Some weeds produce undesirable flavors in milk persisting for 24 hours or longer. About the only way to prevent this type flavor from entering the milk is to completely rid the pastures and feeds of such weeds. Some flavors, such as that of onion, show affinity for the fat portion of the milk and others, such as that of bitterweed, are associated with the skim portion.

Dunkley suggests the following ways to keep feed and weed flavors out of milk:

1. Use feeds that cause no feed flavors.
2. Arrange feeding schedules to prevent cows eating feeds that cause off-flavors during 4 to 5 hours prior to milking.
3. Eliminate weeds from pastures and crops to be used for hay or concentrate.

Flavors Can Be Removed

Equipment is available today that will remove nearly all of the off-flavors caused by feeds and weeds. Alarm has been sounded from some quarters lest such equipment be used as a cure-all for production deficiencies. The alarmists must be assured that such application is not desirable nor is it practical, for every dairy producer knows that highest quality finished products require highest quality raw materials; and that relaxation of the field program on any one point results in a general letdown. The real basis for using such off-flavor removal equipment is to assure day-to-day consistency of good flavor. The realistic approach recognizes that, in spite of great diligence on the part of even the best producer, some feed flavor will occasionally get into the milk, especially if only a few animals of the herd are affected and a pipeline milking system is used. There is a very real problem for the milk receiving inspector in deciding when the concentration is high enough to justify rejection of the milk. Use of the off flavor removing equipment lessens the problems, and borderline to moderate concentrations of feed or weed flavor will not necessitate costly rejection.

The equipment available ranges from simple vacuum chambers through which the hot milk passes during pasteurization to rather complex, automatic, and completely safeguarded units used alone or in connection with regular high-temperature, short-time pasteurizers. The latter units, for the most part, use injection of purified steam with subsequent vaporization under vacuum.

The concept is not new and as pointed out earlier, was used first for removal of undesirable flavors from cream to be used for butter manufacture. Although the mechanism of steam removal is very interesting it will be mentioned here only very briefly.

Murray *(11)* teaches that the volatile flavor substances and those that are soluble can be removed by vaporization and those that are not volatile and those that are more or less insoluble require more thorough treatment, such as steam distillation, which both washes and vaporizes. It has been established by observation of commercial installations that more complete removal of feed flavors results in a more uniform product when steam is used with the vacuum treatment than when the vacuum is used alone.

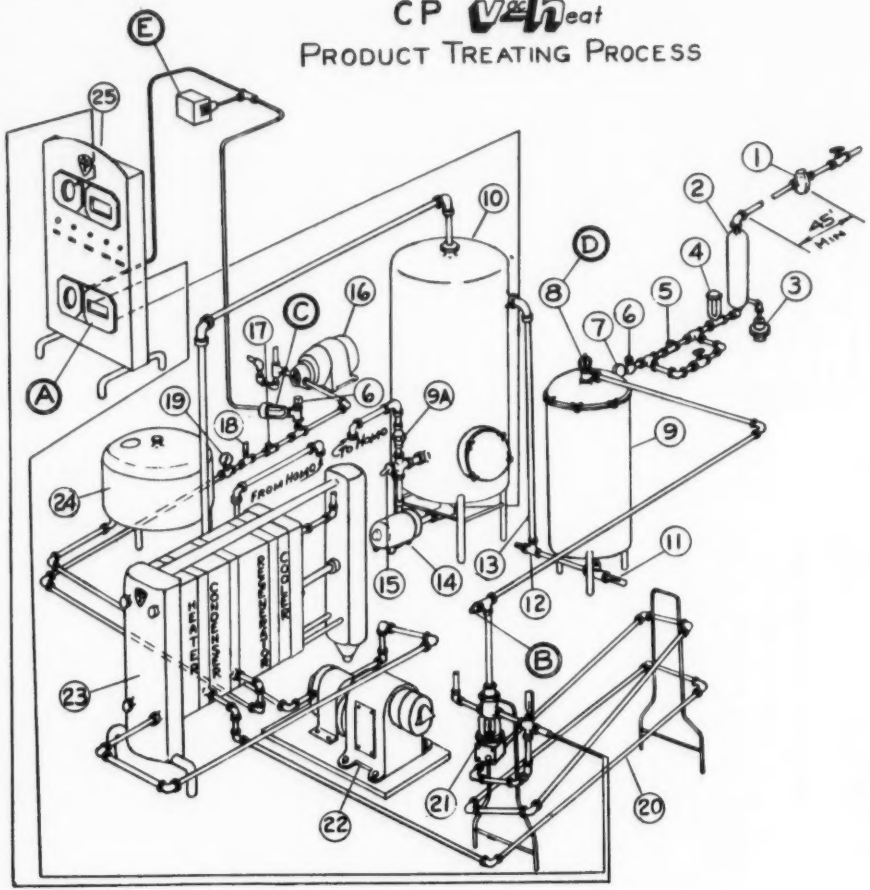
Flavor removal equipment adds to the cost for processing equipment and likewise to operating costs, but the advantages offered through less rejected milk and increased consumption of milk due to more consistent flavor more than offsets these costs. One southern processor had observed that during the summer months his sales dropped. He had assumed that this was due to the hot weather and that many of his customers went on vacations at that time. After installing a steam-vacuum treating unit for feed flavor removal he found, to his surprise, that milk consumption during this period increased. After reviewing this experience, he concluded that he had been having weed and feed flavor during previous seasons and that this new addition to his process gave more consistent flavor, free of these taints. The quality of his product was such that, although his customers did not complain, sales suffered because of decreased consumption.

Typical Equipment

Typical of the modern equipment available today is that manufactured in the United States by the Creamery Package Mfg. Company and the Cherry-Burrell Corporation; and that being imported and distributed by APV and De Laval. Some of these designs use a steam treatment followed by vaporization under vacuum; some use only the vacuum. All may be integrated into a standard high-temperature, short-time system.

A typical steam and vacuum process will be discussed in some detail in order to illustrate the principles of flavor removal, integration into the high-temperature, short-time pasteurizer line, and the controls that permit automatic control of predetermined conditions of operation and make the operation safe and acceptable to public health agencies.

Because the authors are most familiar with equipment manufactured by the Creamery Package Mfg. Company, and in order to avoid confusion, the discussion of a typical system will be confined to that company's process. This process, known as the Vac-Heat Product Treating Process, incorporates the recognized advantages of an HTST pasteurizing unit. The equipment consists of an atomizing cylinder, a vaporizing cylinder, a vacuum pump, condenser, necessary controls, steam purifier and valves.



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9. Atomizing Cylinder
10. Vaporizing Cylinder
11. Thermometer
12. Vacuum Differential Valve
13. Inter-Tank Piping
14. Sanitary Centrifugal Pump
15. Compression By-Pass Valve
16. Rotary Vacuum Pump
17. Check Valve
18. Thermometer
19. Vacuum Gauge
19A Check Valve

The equipment items listed below are shown to indicate a typical existing plate type HTST Pasteurizer to which a vapor condensing section has been added. A separate CP plate type condenser should be used when CP can not add a condenser to an existing pasteurizer installation.

20. Holder Tube
21. Flow Diversion Valve
22. Timing Pump
23. Plate Heat Exchanger
24. Balance Tank
25. Control Panel

SPECIAL SAFETY CONTROLS FOR VAC-HEAT PRODUCT TREATING PROCESS

A. Special Ratio Recorder Controller
B. Vacuum Relief Valve
C. Air Operated Air Throttling Valve
D. Combination Atomizing and Stop Valve
E. Limit Control

HTST Plate Equipment and Accessories to meet USPHS Standards.

The process has been designed with such flexibility that it can be adapted to handling a variety of products and for the removal of various volatile off flavors and odors over a great range of intensities. The amount of heating by steam required in the atomizing cylinder is dependent upon the kind and intensity of the volatile flavors that are to be removed and the efficiency of removal desired. The equipment can be operated with any amount of steam from none, with a product temperature of 161° F. (flow diversion temperature), to 194° F. or even higher. For maintenance of maximum cream line, milk should be subjected to the minimum steam treatment consistent with removal of the feed or weed flavors. The temperatures and pressures in the following discussion are typical for a homogenized milk process and do not necessarily apply to any individual installation.

The product flow is through the raw side of the regenerator to the heater and holding tube of a standard HTST pasteurizer, then from the flow diversion valve through an atomizing nozzle and into the atomizing cylinder where it falls through a steam vapor atmosphere to be heated to 194° F. under approximately 9 in. of vacuum. From this cylinder it is drawn through a pressure differential valve to the vaporizing cylinder. There it is subjected to controlled vaporization under 22 in. of vacuum. The milk is pumped from the vaporizing cylinder through the pasteurized side of the regenerator and the cooling section of the HTST system from whence it flows to the prefiller surge tank to await packaging or bottling.

The product is heated to the steam vapor temperature in the atomizing cylinder as it falls through the vapor to the bottom of the vessel. An excess of vapor assures that each particle of milk from the atomizing nozzle is uniformly heated. The temperature of the milk in the atomizing cylinder is governed by the vacuum as regulated by the adjustment of the vacuum differential valve.

A safety device is installed between the atomizing nozzle and the flow diversion valve which prevents a vacuum from being pulled on the flow diversion valve when it is in the diverted position. If a vacuum were to be applied while the plug is in the diverted flow position, there might be a tendency for a speed-up in milk flow in the holder at the moment of resuming forward flow, which might reduce the holding time below prescribed limits.

The product remains at the high temperature for only a very brief time, for as it passes through the vacuum differential valve it is flash cooled to 152° F. under approximately 22 in. vacuum. The milk enters the vaporization cylinder through a tangential opening and swirls around the sides as it drops to the bottom of the cylinder. The water vapor and noncondensable gases are removed off the top of the cylinder through the condenser, which, in the Vac-Heat unit, is a separate, water-cooled section of the plate heat exchanger making up the HTST pasteurizer. The plate arrangement of the condenser section is engineered so that the condensate drains by gravity out of each flow stream, thus obtaining the full plate area for condensing surface. The water requirements for condensing amounts to approximately 1 gal. per minute for each 1,000 lb. per hour product capacity. A 10,000 lb. per hour unit would require about 10 gal. of water per minute with a temperature rise of 80° F.

Steam requirements are approximately one boiler horsepower per 1,000 lb. of product when heating from 162° F. to 194° F. Refrigeration requirements, on the other hand, are actually reduced to a small extent.

Safety controls prevent product dilution or concentration by maintaining a set temperature differential between the milk entering the Vac-Heat equipment from the flow diversion valve and the milk leaving the vaporizing cylinder. The temperature differential used assures that the heat added just balances the heat removed by the second cylinder plus that lost through the surfaces of the equipment. A specially designed ratio recorder-controller is used for the maintenance of the temperature differential. This control operates the flow diversion valve to divert the milk flow if the temperature in the vaporizing cylinder exceeds the pre-set operating temperature. It automatically adjusts the vacuum below this point to produce the operating temperatures equivalent to settings made at the time of equipment installation.

Operation is automatic. There are no adjustments required to put the equipment in forward flow from diverted flow, for as soon as the operating temperatures are obtained, forward flow is automatically resumed.

Although the equipment manufactured by the various concerns is intended to perform the same function of off-flavor removal, there are differences in design details and in control equipment which affect the original cost, ease of operation, economy of operation, and thoroughness of removal of undesirable flavors and odors.

Summary

The problem of undesirable weed and feed flavors in milk is world wide. Equipment is now available with advanced controls which is capable of almost complete removal of these odors; which assures uniformity of milk flavor; and which is acceptable to public health agencies. The use of such equipment in the pasteurizing line results in a product having better consumer acceptance, an important factor in increasing the consumption of milk and milk products.

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Making the Most of Dairy Farm Records

How John Davis, A Young Michigan Farmer, Achieved Success

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In this age of mechanized farming there has been a tendency to place major emphasis on the attainment of high output per man, per animal, and per acre. Relatively little attention has been given to the importance of time spent in keeping useful records and in analyzing and planning the farm business.

Reducing the daily time required to care for a cow does not in itself increase the net income of a dairy farmer. The dairy farmer needs to determine the investments and costs of reducing labor requirements and the alternative uses of the labor saved before he can make a critical appraisal of this change. Likewise, attaining high production per cow is not in itself a guarantee of high net returns. Perhaps this high production was obtained by feeding grain at unprofitable rates. Meaningful records provide a realistic basis for analyzing the production records on an individual farm.

Dairymen Must Establish Useful Records

A dairy farmer without records has been likened to a clock without hands. The dairy farmer may be just as confused about his business as the person who tries to guess the time of day by looking at the handless clock. However, the mere keeping of records is not enough. These records need to serve a useful purpose. They should help a dairy farmer to benefit from past mistakes as well as successes. They should help him select the best alternatives when he is considering a change in technique or method. They should help him to minimize losses or maximize returns with changing price relationships and should help him and his family to balance out expenditures between the farm business and the home. This becomes very important if the farm business is to prosper and if the goals of the farm family are to be attained.

John Davis, a 41-year-old Eaton County, Michigan, dairyman has been successful in attaining these and other benefits of farm record keeping. His records have paid dividends in higher net income, better living, greater ease in doing farm work, and satisfactions in a job well done.

Davis Develops Successful Business

Some dairy farmers spend a lifetime in building up a successful farm business. Other dairymen reach this goal in half the time.

John Davis is a dairy farmer who has attained an enviable record while still a comparably young farmer. He has more than doubled milk output in the last 10 years. Milk production per cow has been increased by 30% and cow numbers by 90% during this period.

FIG. 1. High-producing cows, an adequate size herd, and economy in feeding grain and roughage have resulted in high net returns for the Davis family. Useful records have played an important part in attaining these benefits.

There has been no change in farm size. However, the tillable acreage has been increased by clearing and draining 20 acres. A total milk output of 485,700 lb. was reached during 1955. This production was obtained from 42 Holstein cows which averaged 11,710 lb. of 3.5 test milk. Total milk production was increased by 30% but production per cow was reduced by 11% from 1954 to 1955. This was a period in which cow numbers were increased from 28 to 52. John believes that he has now reached a peak in cow numbers. He expects to regain the loss in production per cow by closer culling during 1956.

The labor force on the Davis farm includes John, a son Aaron, who is 16 years old and a sophomore in high school, and 8-year-old twin sons, Jon and Don. Mrs. Davis and a daughter Hazle, aged 13, take care of the household operations. Mrs. Davis also helps John with his record work, in filing farm pictures and in operating a tractor when hay is unloaded at the barn.

Aaron has assumed a major role in the feeding and milking operations. His father has worked out an arrangement with him whereby he receives 10% of the net receipts. He owns 10% of the cows and equipment and pays 10% of all operating expenses. A $1,500 equity in the livestock and equipment has already been acquired. Future savings may be used to pay for a college education, to acquire a larger share of the farm business or for other purposes. The twins help with the chores on every other evening. When these boys become older, they will also be given an opportunity, if they are interested, to purchase a share in the farm business.

FIG. 2. John Davis and son Aaron calculate the net income each one will receive from the farm business. The Davis family uses a combination of records that are useful in analyzing each phase of the farm business.

Economical Feed Production Is First Essential

A first step in developing a successful dairy enterprise is the economical production of feed. A complete field diary has helped John Davis to increase feed production to such an extent that he could greatly increase cow numbers. John records the quantities of all lime, fertilizer, and seed applied to each field and the dates of application. The results of all soil tests are also recorded. He makes notes of the dates crops are planted, cultivated, and harvested. He can tell the yields of all crops by fields and relate these yields to the cropping practices used and weather conditions prevailing during the year.

Careful planning has resulted in a rather unusual rotation of corn—corn—corn—summer fallow and new seeding—alfalfa-brome—alfalfa-brome—alfalfa-brome. This rotation provides for the maximum acreage of high yielding feed crops and the opportunity to establish good seedings. Oats, a low-value crop, has been grown only twice in the last 20 years. All small grain equipment has been eliminated.

Alfalfa and brome grass are seeded with ½ bu. of oats in early August. The oats and new seeding are pastured for 15 to 20 days during November when there is little danger of injury to the stand. This late pasture is usually worth more than an oats crop and has the further advantage of costing nothing to harvest. The second-year corn is harvested for silage with the land immediately plowed and planted to rye. The rye is pastured both fall and spring and then plowed down for corn.

Hay has averaged 4 tons and corn 70 bu. per acre on this farm in recent years. John Davis applied 500 lb. of 5-20-20 fertilizer per acre when new seedings are made and 300 lb. of 0-20-10 each year thereafter. Maximum production will be needed from the 177 acres of cropland if sufficient roughage is to be produced for 52 cows plus the replacement heifers. Davis is considering the investment in an additional silo. His figures show that he can produce 33% more feed per acre when corn is harvested for silage rather than grain. Costs of producing 100 lb. of feed nutrients would be reduced by 20%.

More Cows vs. Higher Producing Cows

Dairy farmers usually find it difficult to increase the numbers of cows and production per

cow at the same time. Good use of records has helped John Davis to achieve better than average success in doing this (Figure 3). He has selected sires from cow families that have shown consistently high production.

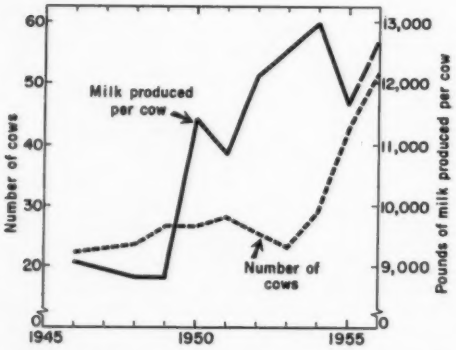

FIG. 3. John Davis has achieved better than average success in increasing both the number of cows and the production per cow at the same time. Good records have been very helpful in attaining this result.

Davis makes good use of D.H.I.A. and other records in deciding which cows to keep and which ones to cull. A heifer that has finished a rather unsatisfactory record is often given a second chance to prove her merits if she comes from a family of good producers. Very often this heifer does prove her worth as a good producer in later lactations.

Persistency of production during the lactation period is another factor that John Davis checks rather closely in selecting which cows to keep. A cow often produces well for the first 4 or 5 months and then drops off sharply in production. A comparison was made of the production records of three cows which started out the lactation by milking from 62 to 71 lb. daily. Two of these cows produced 40 to 50% more milk than the third one during the entire lactation period. John prepares tables similar to the one in opposite column, to show how well individual cows produce.

Month in lactation period	Cow No.		
	136	156	198
	(lb. milk produced daily)		
1	67	62	71
2	55	51	67
3	51	37	61
4	40	41	43
5	31	42	50
6	23	37	59
7	20	36	52
8	11	44	39
9	07	31	23
10	—	—	05
Total production 3.5 test milk	9,880	13,570	14,630
Days in lactation	281	291	312

Controlled Freshening Pattern Increases Net Income

Dairymen who sell milk on a base-surplus plan usually find it profitable to shift freshenings to a fall basis. Most dairymen experience difficulty in getting cows to freshen at the time of year which results in a high proportion of the milk selling at a base price. The 5-month period August through December is the time when Davis and other Michigan dairy farmers have the opportunity of establishing a daily base for the next 12 months.

During 1955, 49 out of his 50 cows freshened during the period July 15 through December 31. Eighteen of these cows freshened in July and 12 in August. Milk production is at a high level from August through April and drops to a low during June and most of July (Figure 4).

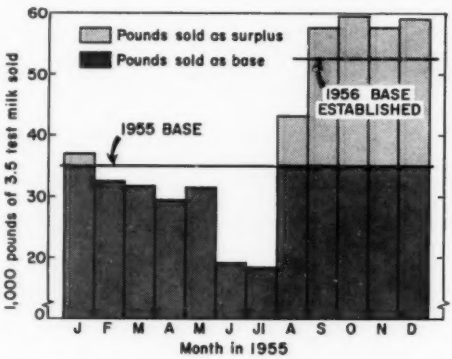

FIG. 4. Over 90% of the milk sold on the Davis farm brings the higher base price. The number of cows milked and the total milk produced is at a low point in June and July when milk prices are lowest and when labor demands for hay harvesting are at a peak.

The number of cows milked is at a low point when labor demands for hay harvesting are at a peak. After haying operations, there is usually some time left for a short vacation before fall freshening begins.

In recent years, 90% or more of the milk produced has been sold at the base price. The daily base established annually for this farm was increased from 460 lb. for 1943 to 1,752 lb. for 1956. Base milk has sold from $0.80 to $1.00 or more higher per cwt. for 3.5 test milk than has milk designated as surplus. Davis has plotted the monthly production of milk along with the base established for every year since 1943. In 1947, he started to show the monthly freshening pattern. These graphs have been very helpful in the breeding and culling program. The freshening pattern attained on this

farm has added from $1,200 to $1,600 to gross income per year over what it would have been for a more average pattern.

John Davis depends on fall freshening heifers to help maintain a high base production. Since most of the heifers are born in the fall, it becomes necessary to grow them out rapidly so they can freshen when they are 2 years of age. In the past, he has kept some heifers over to freshen at 2½ to 3 years of age. He cannot afford to do this now because of the large numbers of cows kept.

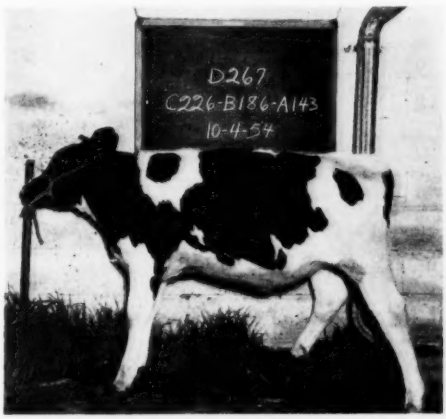

FIG. 5. John Davis photographs each heifer calf born on the farm. A blackboard hung on the wall shows heifer number D267. Three generations of ancestors and date of birth are lettered in below. The capital letters A, B, C, and D refer to the four herd sires that have been used. The numbers identify the maternal ancestors. This photograph forms a permanent record of identification.

Calves receive whole milk for an 8-week period, starting with 8 lb. daily and dropping to 2 lb. daily for the last 2 weeks. The first 6 months, heifer calves are self-fed a grain mixture consisting of 20% pellets, 40% ground oats and 40% ground shelled corn. The next 6 months, calves are fed an average of 7 lb. daily of a grain mixture testing 15% protein. From then until the heifers freshen, they are fed 5 lb. of ground ear corn daily. During this entire period, heifers are fed some of the best quality hay Davis produces. This is a change from his previous practice of feeding the milking herd the best roughage with the heifers receiving the poorer quality hay.

The table below shows the kind of information John Davis obtained on six heifers that will freshen in July.

These heifers will be well grown out when they freshen. The additional costs of growing out heifers appear to be good business for John Davis.

Attaining a Satisfying Farm and Family Relationship

Are detailed dairy farm records worth the time and efforts they require? The John Davis family says yes. Thirty minutes a day spent in recording and analyzing their farm activities

FIG. 6. Detailed dairy farm records have paid off in a better farm living, a debt-free farm, less drudgery in farming and the opportunity for all members of the Davis family to participate in farm and home activities.

have yielded the following dividends: higher net income, a debt-free farm, an open-mindedness to try new farming methods, less drudgery in farming, setting high standards of achievements for the children, opportunities to include the entire family in all activities, and a personal satisfaction in achieving success.

The use of a stop-watch in timing alternative work methods has shortened the time needed to feed and milk the cows. Either John Davis or his son Aaron can perform these operations in 2 hours. The two of them working together with

Heifer No.	Age when bred	Age when due	Weight when bred[a]	Weight on April 28[a]	Date due
	(mo.)	*(mo.)*	*(lb.)*	*(lb.)*	
262	14.7	23.7	875	1,160	7-21
263	14.7	23.7	910	1,240	7-24
264	14.3	23.3	880	1,190	8-10
265	14.5	23.5	810	1,060	7-30
266	14.0	23.0	820	1,140	7-16
267	12.5	21.5	715	1,010	7-23
Av.	14.1	23.1	835	1,133	7-25

[a] Taped weight.

the help of one of the twins can complete these chores in considerably less time. The conversion to a loose-housing system, the use of overhead bins and elevators, and other labor-saving methods have made farm life more pleasant. An inter-com system allows Mrs. Davis to call John at the barn to remind him that it's meal time or that a visitor has just arrived.

The Davis family finds time to participate in community affairs. John is a board member of the Battle Creek Milk Producers Association and secretary of the local school board. Mrs. Davis belongs to the Walton Center Extension group and is secretary of the church board. Aaron was recently elected secretary of the local chapter of the Future Farmers of America. He was the recipient of the local F.F.A. award for the best dairy program. This award was based largely on his ingenuity and accomplishments in caring for the dairy equipment and cows.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

514. Controlling mastitis increases income. J. E. EDMONDSON and C. P. MERILAN, Univ. of Mo., Columbia. Milk Plant Monthly, **45**, 5: 37. 1956.

In 1955, mastitis cost the American dairy farmer an estimated $226,000,000. Approximately 25% of the dairy cows are infected with mastitis. This results in a 15% increase in herd replacements. Mastitis also causes a 15 to 20% decrease in milk production. It is recognized as acute, sub-clinical, and chronic. Its diagnosis and treatment are discussed.

C. J. Babcock

515. The sensitivity to chemotherapeutic agents of a further series of strains of Escherichia coli from cases of white scours: The relationship between sensitivity tests and response to treatment. H. W. SMITH and W. E. CRABB, An. Health Inst., Stock, Essex. Vet. Record, **68**, 19: 274. 1956.

Fifty-eight strains of *E. coli* isolated from feces of different cases of white scours in calves were studied in vitro for sensitivity to chemotherapeutic agents. These results are compared to a similar study made from 1950-53, and before extensive use of some of the newer chemotherapeutic agents. The sensitivity of the 58 cultures to sulphadimidine, dihydrostreptomycin, chloramphenical, chlortetracycline (aureomycin), and oxytetracycline (terramycin) was measured. A higher proportion of strains was resistant to one or more of these agents in this survey than in the 1950-53 survey. This was especially true of streptomycin, the commonly employed agent for the treatment of white scours in the period between the surveys. Aureomycin and terramycin resistant strains were found only on 2 farms which had routinely fed aureomycin feed supplements for several years. The authors discuss the dangers that may accompany prophylactic chemotherapy of white scours. R. P. Niedermeier

516. Congenital infection with Mycobacterium johnei in cattle. W. E. LAWRENCE, Min. of Agr., Vet. Investigations Center, Liverpool. Vet Record, **68**, 21: 312. 1956.

Possibility of congenital infection of the calf with *M. johnei* was studied with 24 bovine fetuses, most of which were obtained in utero from cows slaughtered with a history of Johne's disease. Cultures of organisms resembling Johne's bacillus were obtained from 5 of the 24 fetuses examined. R. P. Niedermeier

517. Milk fever. A. ROBERTSON, A. MARR, and E. W. MOODIE, Royal School of Vet. Studies, Univ. of Edinburgh. Vet. Record, **68**, 13: 173. 1956.

Various investigations are included in this report. Changes in serum calcium and magnesium, and blood inorganic phosphate are given for 32 normal calvings. Included are cows calving for the 1st, 2nd, 3rd and 4th or more times. Calcium and inorganic phosphate fell 11.7% and 33.8% respectively for 1st calvings, and increased to 30.6% and 48.1% for 4th or more calvings. Serum magnesium increased from 7.7% for 1st calvings to 35.9% for 4th or more calvings.

Prepartum milking of 3 cows only slightly influenced the blood levels of calcium, phosphorus and magnesium at parturition. Three pregnant mastectomized cows showed only a slight change in serum calcium and no change in serum magnesium at parturition. However, plasma phosphate levels showed decreases similar to normal calvings. R. P. Niedermeier

BUTTER

518. Milk to butter or fat—in uninterrupted flow. H. DAVIS and P. C. ROAHEN, Creamery Pkg. Mfg. Co., Fort Atkinson, Wis. Food Eng., **28**, 4: 50. 1956.

Rice Lake Creamery Co., Rice Lake, Wis., is utilizing a continuous system for butter manufacture and/or producing anhydrous milk fat. Capacity is 2000 lbs. butter/hr. starting with raw milk.

Milk at 90° F. is separated to 40% cream, which is heated to 195° F., cooled to 165° F. and reseparated in a heavy cream separator to 80% butterfat. The heavy cream goes to a phase reverser and then to a serum separator. The resulting 98.5-99% fat product is heated to 190° F. and then cooled to 110-120° F. The

high fat product flows to the composition control vats and is standardized for fat, moisture, salt, and curd content. The butter-mix is cooled to 40° F. by a chilling unit and then goes to the crystallizer. The butter emerging from the crystallizer may be handled by a printer or a box filler.

The process for anhydrous fat is the same up to the serum separator. The 98% fat then goes to the oil polisher which further reduces moisture and serum solids. After additional treatment in a vacuum pan the fat content is raised to 99.8%. The fat is then cooled to 110-120° F. and finally run through the chilling unit. Among various advantages cited for the system are reduction in labor, improved sanitation, and more precise composition control.

T. J. Claydon

519. Yield and properties of fractions separated from buttermilk by cold crystallization. K. G. Weckel and J. A. Stein, Univ. of Wis. The Milk Dealer, **45**, 9: 154. 1956.

Butterfat was separated into four fractions by means of cold fractional crystallization. The yield of the various fractions was greatly affected by the season in which the butterfat was produced and by the type of ration which the cows received. It was shown by means of penetration and dilation measurements that the butterfat and the fractions separated from it differ much in consistency and in melting characteristics. Similarly the degree of unsaturation of the fat in the fractions was shown to vary. Data is presented showing the average yields of fractions separated by crystallization and estimated percentage solid glycerides in whole "winter ration" butterfat and its fractions at various temperatures. C. J. Babcock

CHEESE

520. Streamlined production and packaging of cottage cheese. A. G. Hale, Paper Cup and Container Institute, N. Y., N. Y. Milk Dealer, **45**, 8: 50. 1956.

A description of the streamlined production and packaging assemblies for cottage cheese used by the Borden's Farm Products Co. plant in Long Island City, N. Y. At this plant 8 oz. containers are filled at the rate of 65 per min., and 16 oz. containers at the rate of 50 per min.

C. J. Babcock

CONDENSED AND DRIED MILKS; BY-PRODUCTS

521. Skillful processing broadens food uses for modern whey solids. J. V. Ziemba. Food Eng., **28**, 6: 74. 1956.

A stabilized, free flowing whey powder is produced by Western Condensing Co., Appleton, Wisconsin, by first crystallizing the lactose in condensed whey and then drying. The operation involves centralized drying of whey from a number of outlying cheese factories. The whey is heated to 220° F. and concentrated in a 4-effect evaporator to 28-30% solids. Further concentration to 50% solids is accomplished in a single effect evaporator. The concentrate is stabilized by cooling with agitation which accelerates lactose crystallization. The product is spray dried to 15% moisture and further dried in a hot-air vibrating dryer to 4-5% moisture.

Different whey products are produced, including coarse and fine powders, products modified with NFDMS and/or soya solids, and a whipping agent. The dried whey products are used in cheese foods, baked products, coatings, fillings, candies, ices, sherbets, and ice cream.

T. J. Claydon

522. Official grading of nonfat dry milk solids. T. I. Hedrick and B. J. Ommodt, A.M.S., U.S.D.A. Milk Prod. J., **47**, 5: 13. 1956.

The authors discuss the importance of governmental grading of nonfat dry milk solids. During the past several years ½-¾ of a billion lbs. of nonfat dry milk solids have been graded each year by the Inspection and Grading Laboratory, Dairy Division, Agricultural Marketing Service, U.S.D.A. The grading of NDMS is confined to chemical, bacteriological and organoleptic analyses of taste, sight and touch. Agreement between plants and laboratory are frequent and help considerably in maintaining quality. Variations occur most often with moisture tests and scorched particle tests. However, the use of identical techniques helps to minimize these variations. J. J. Janzen

DAIRY BACTERIOLOGY

523. Incidence of lactic starter inhibitory substances in individual herd milks of Wisconsin. C. E. Neal and H. E. Calbert, Univ. of Wis., Madison. Milk Dealer, **45**, 8: 49. 1956.

A survey to determine the incidence of lactic starter inhibitors in the herd milks delivered to a total of 30 dairies showed that residual antibiotics in the milk supply does not appear to be a widespread problem in Wis. The milk samples were tested by a modified started activity test, using a mixed strain lactic starter as the test culture, and the 2,3,5-triphenyltetrazolium chloride (TTC) test, employing *S. thermophilus* as the test culture. Data are presented showing the characteristics of individual herd milks when tested by the TTC test. The number of herd milks that showed strong inhibition was 2.4% by the TTC test and 0.4% with the starter activity method. This indicates that some producers are not following the necessary recommend practice of withholding the milk from a treated quarter for 72 hrs. (6 milkings) after an antibiotic treatment.

C. J. Babcock

524. Improved starter cultures. ANON. Milk Prod. J., **47**, 6: 16. 1956.

Research at Oregon State College has elucidated the nutritional requirements of lactic streptococci from the standpoint of fundamental requirements, factors affecting growth in milk, and finally, variations in content of growth stimulatory peptides in different samples of individual cow's milk.

Results have shown that the peptide content of milk varies widely between cows. This variation affects the ability of fresh, whole milk to support the rapid growth of lactic acid bacteria. The peptides tend to stimulate the bacteria in the early growth phase where vigor is most needed to suppress undesirable bacteria.

J. J. Janzen

DAIRY CHEMISTRY

525. Incidence of lactic starter inhibitory substances in individual herd milks in Wisconsin. C. E. NEAL and H. E. CALBERT, Dept. Dairy & Food Ind., Univ. of Wis. Milk Prod. J., **47**, 6: 18. 1956.

The authors discuss the results obtained in the survey conducted to determine the presence of starter inhibitors in Wisconsin milk.

The number of herd milks that showed strong inhibition of lactic starter was 2.4% by the T.T.C. test and 0.4% with the starter activity method. These results indicate that some producers are not withholding the milk from a treated quarter for 72 hours after antibiotic treatment. The authors conclude that residual antibiotics in the milk supply is not a widespread problem in Wisconsin. J. J. Janzen

526. An economical semi-mechanical stirrer and tapper for the cryoscope. W. L. GREEN, Ind. State Dept. of Health. Milk and Food Technol., 19, 192. 1956.

A semi-automatic sample stirrer and thermometer tapper has been developed to determine the freezing point of milk. The av. of the differences on the freezing point of 18 raw milk samples was 0.0026° C. The minimum was 0.000° C. and the maximum difference was 0.007° C. H. H. Weiser

DAIRY ENGINEERING

527. New developments in the use of separators and clarifiers. K. PISTORIUS, DeLaval Separator Co., Chicago, Ill. S. Dairy Prod. J., **59**, 2: 48. 1956.

The use of air tight separators and clarifiers has aided in eliminating air from milk during processing, improved the quality of the cream and increased the efficiency of clarification. The cold milk separator has resulted in savings in equipment and in eliminating heating and cooling and in improvement in quality and viscosity of cream. Cold clarification is recommended generally to avoid getting some of the sediment into solution. The richness of milk from a standardizing clarifier may vary with the richness of the original milk and its temperature. Operation of separators or standardizing clarifiers should be continuous to avoid the concentration of cream in the center of the bowl. F. W. Bennett

528. Insulation in truck bodies. J. M. DANIEL, JR., Hackney Bros. Body Co., Wilson, N. C. Milk Dealer, **45**, 8: 106. 1956.

The features required or desirable on insulants for application in a low or medium temperature truck body are: low conductivity, low weight, low heat capacity, low moisture absorption, high water vapor resistance, low installed cost and long lift. Other factors to be considered are: vermin resistance, odor, odor absorption, toxicity, and availability. Still other items are structure to support, fire resistance, chemical resistance, and compressive strength. These features are discussed as they relate to: 1. Mass (Fill Type) insulation. 2. Foam type insulants, and 3. Reflective insulants.

C. J. Babcock

529. Economies in steam and refrigeration in dairy plants. C. W. HALL, Mich. State Univ., East Lansing. The Milk Dealer, **45**, 9: 55. 1956.

A reasonable figure for the cost of steam up to 100 psi. based on equipment, labor and fuel prices is $3 per 1,000 lbs. Many dairies pay less and a few pay more. For a 40,000 lbs. per day plant the cost of refrigeration based on today's equipment prices would be $.06 per ton of refrigeration for each hour. Savings are made throughout a plant if the efficiency of producing steam and refrigeration is increased. Then reduce the need for these services wherever possible, such as HTST pasteurization, cold milk separation. Then study each individual operation to locate sources of energy losses. The principles are the same whether steam or refrigeration is involved. If the plant operations are observed critically, costs can be reduced and income increased.

C. J. Babcock

530. Corrosion protection of steel. A. G. STERNBERG, Steelcote Manufacturing Co., St. Louis, Mo. The Milk Dealer, **45**, 9: 150. 1956.

Epoxy finishes have become prominent due to the great versatility of these new resins. They have chemical resistance, adhesion and flexibility. Their basic raw materials can be derived from petroleum. The internal structure of epoxies contain "ether" type linkages instead of the "ester" type usually found in protective coatings, and the "ether" type is the most stable known to organic chemists. They have excellent cohesion and yet tremendous adhesion. Their adhesion exceeds their cohesion and even when peeling is manually

started they cannot, normally, be stripped from a panel. They are fairly light colored and may be colored with almost any chosen pigment. The five basic paint formulations in the epoxy group are: (1) Esters, (2) Amines, (3) Polyamides, (4) Ureas, and (5) Phenolics. For maximum corrosion resistance to water, salt, acid or alkali, and on a surface which may be allowed to dry, the finish should consist of a rust inhibitive coat, a moisture barrier coat, and a decorative coat. C. J. Babcock

DAIRY PLANT MANAGEMENT AND ECONOMICS

531. Installment financing of capital equipment. S. D. Maddock, Pres. C.I.T. Corp. Milk Prod. J., **47**, 5: 10. 1956.

The author discusses the merits of installment buying of needed machinery versus the older methods such as cash payment, short-term loan, or credit from the supplier.

Terms must fit the individual needs of each case and a second financing schedule will permit the new machinery to pay for itself.

J. J. Janzen

532. Installment financing of capital equipment. S. D. Maddock, C.I.T. Corp. Milk Dealer, **45**, 8: 54. 1956.

When the new faster depreciation schedules were placed in the tax laws, a new approach to installment financing designed to take advantage of the new depreciation methods was introduced. It is known as "Pay-as-You-Depreciate" method of financing and answers just about all the needs of an equipment buyer. It permits the buyer to use depreciation reserves for the payments in a way he could not do previously because payments parallel depreciation allowances. The sum-of-the-digits method of depreciation chosen for this plan is described. It allows the fastest depreciation.

C. J. Babcock

533. Icing of milk crates. J. Dressel, Dressel-Young Dairy, Granite City, Ill. Milk Dealer, **54**, 8: 90. 1956.

The icing of milk crates is not only simplified but safer with machine-made flakes. The initial cost involves some extra expense but once it is paid for, there is little expense involved. A typical flake or chip ice-maker can produce from 8 to 25 tons of flake ice a day. It fits into a space approximately 12 ft. by 6 ft. The construction and operation of this equiment is discussed. C. J. Babcock

534. Consumer preferences for dairy products and services in Oklahoma City, L. V. Blakely, L. D. McMullin, and K. B. Boggs, Okla. Agr. Expt. Sta. Bull., B-464. 1955.

Through a survey of 1% of the population of Oklahoma City (821 households) it was found that 95% used fluid milk; that less than 20% used fluid cream; that filled milk represented about half the canned milk sales; that about three times as many families used oleomargarine than butter; and that about twice as many families used ice cream than mellorine. Data are also presented concerning delivery preference, type of container, and other consumer preferences. A. W. Rudnick, Jr.

535. An interim report on milk marketing distribution systems in Ohio. G. H. Mitchell and E. F. Boumer, Ohio Agr. Expt. Sta. Res. Circ., 29. 1956.

This preliminary report of an investigation of fluid milk marketing describes the per capita consumption, trends of delivery and consumer preference for container size and type. Five Ohio markets have been studied, considerable variation in most factors considered was found when comparisons were made between markets.

A. W. Rudnick, Jr.

536. Dairy substitutes in Missouri. S. F. Whitted, Mo. Agr. Expt. Sta. Bull., 658. 1955.

Three hundred and twenty-nine persons in Missouri were interviewed concerning their use and preference for vegetable fat frozen dessert, filled evaporated milk and "aerated" cream. The largest number finding the vegetable substitute for ice cream "satisfactory" appeared in the age groups 16 and under, with the preponderance at the 12 year level. Most respondents seemed to allow price to influence their definition of the term "satisfactory."

A. W. Rudnick, Jr.

537. Twin quart packaging. E. Thom. The Milk Dealer, **45**, 9: 44. 1956.

A new multiple qt. unit, two single units wrapped together by a band and including a convenient carrying handle, proved highly popular with Eugene, Ore., consumers and enabled the Echo Spring Dairy to meet ½ gal. competition without making a major change in plant packaging equipment. The twin qt. unit increased qt. sales on wholesale routes 55% and qt. sales on retail routes 34.5%.

C. J. Babcock

538. Bulk hauling—its cost problems—its savings. A. S. Henderson, Arden Farms Co., Seattle, and R. Cowan, Seattle Milk Producers Assoc. Milk Plant Monthly, **45**, 5: 18. 1956.

The use of bulk farm tanks has made the cost of stopping to pick up milk a more significant part of the hauling cost than the distance the milk is hauled. It has further reduced the importance of distance between the farm and the plant. A combination charge of a cost per cwt. and a cost per stop or pick-up is used by some milk processors. Hauling costs, however, have been reduced by bulk pick-up.

Figures are presented showing an average saving of .0529 per cwt. in the hauling of all milk, with a reduction of .0728 per cwt. for tank, and an increase of .0420 per cwt. for can milk. The cost of hauling milk in cans has been raised as much as 5¢ per can in certain districts. C. J. Babcock

539. Fast pace in all-bulk conversion. M. MICHAEL. Milk Plant Monthly, **45**, 5: 21. 1956.

A year ago the Twin City Milk Producers Assoc. announced plans for 100% conversion to bulk-pick-up. Almost 700 farm tanks and 25 tank trucks are in operation. They represent 25-30% of the assoc. members' milk production. The objective of 100% conversion may be completed in 1957. They are using 12 2,300 gal. and 13 1,700 gal. capacity tank trucks. Installation costs per tank have been averaging less than the original estimate. Producers are buying more capacity in their tanks than they presently need. Of 1,529 farms the capactities ordered are: 2.5% 100 Gal.; 8.8% 150 Gal.; 26% 200 Gal.; 41.3% 300 Gal.; 14.2% 400 Gal.; 3.9% 500 Gal.; 2.3% 600 Gal.; and 1% 700 Gal. C. J. Babcock

540. Thinking about financing bulk farm plants. C. E. FRENCH, Purdue Univ., Lafayette, Ind., Milk Plant Monthly, **45**, 5: 28. 1956.

A big investment is necessary in making the change to farm tanks. Annual use cost on the investment includes the normal depreciation rate and the so-called hidden costs of interest, insurance, repairs and taxes on the tanks. These equal about 5% of the initial investment. A tank installation that costs $2,000 and lasts 10 years may well commit a farmer for an annual cost of $300 per year. Savings per cwt. vary among markets. Among the sources of savings to consider are: (1) Direct farm savings (cans, milk saved, test difference, labor, operating costs, etc.), (2) Indirect farm savings (ease of work, better quality, better management, etc.), (3) Hauling rate reductions, and (4) Dealer premiums. C. J. Babcock

541. Dairy and producers hail switch to bulk. ANON. Milk Dealer, **45**, 8: 52. 1956.

It required 5 mo. for the Quality Dairy, Willmar, Minn. to convert from cans to bulk. Each of the farms from which the dairy purchases milk has been equipped with a refrigerated holding tank. The tanks are self-liquidating. The dairy pays a 10¢ per cwt. premium to its producers and also hauls the milk for 5¢ per cwt. less in bulk. It deducts 15¢ per cwt. from each producer's check and adds its own 15¢ per cwt. figure. This makes a 30¢ per cwt. payment on the tank. The dairy discontinues the 10¢ premium and charges the farmer 15¢ per cwt. for hauling after the tank is paid for. It requires about 3 yrs. for the av. producer to own his tank. C. J. Babcock

FEEDS AND FEEDING

542. The feeding value of alfalfa for dairy cows. E. A. KEYS and E. P. SMITH, Mont. Agr. Expt. Sta. Bull., 513. 1955.

Feeding trials were conducted using alfalfa from the same field put up as baled hay, chopped hay, loose hay, and silage. Although the av. milk production of cows fed chopped hay was the highest, those fed silage produced the greatest amount of F.C.M. per lb. of T.D.N. and per lb. of protein. The feed cost per 100 lb. of F.C.M. from silage was 50¢ less than that for any of the other forms of forage. All cows were fed grain at the same rate and gained weight during the 120 d. test period.
A. W. Rudnick, Jr.

543. A preliminary evaluation of dicyandiamide and urea for dairy cattle. C. A. LASSITER, D. M. SEATH, F. B. HAMBLIN, B. M. ADAMS, and G. M. BASTIN, Ky. Agr. Expt. Sta. Bull., 638. 1955.

Feeding trials with lactating dairy cows and a standard digestion trial with dairy steers indicated that urea, dicyandiamide, and soybean oil meal were of comparable value for milk production. The urea and dicyandiamide were substituted for one-third of the protein in the concentrate mixtures of dairy cattle. While there was an indication that the feed mixtures containing these compounds were slightly less palatable, digestion trial data indicated very little difference in digestibility of the three mixtures. A. W. Rudnick, Jr.

544. Relative efficiency of three levels of concentrated feeding for milk production. J. R. OWEN, R. T. COUMPTON, and J. T. MILES, Miss. Agr. Expt. Sta. Inf. Sheet, 518. 1955.

When milk is priced at $4.70 per cwt., feeding grain at a ratio of one lb. of concentrate per three lbs. of milk is the most economical feed ration. At manufacturing milk price of $3.00 per cwt., it is more economical to feed one lb. of concentrate for each six lbs. of milk.
A. W. Rudnick, Jr.

545. Ruminant nutrition. In vivo and in vitro nutritional requirements of rumen microorganisms. R. R. JOHNSON, O. G. BENTLEY, J. W. HIBBS, and H. R. CONRAD, Ohio. Agr. Expt. Sta., Wooster. J. Agr. Food Chem., 4: 627. 1956.

The composition of the ration influences the digestive and synthetic processes of rumen microorganisms. Both rumen inoculations and changes in the ratio of roughage to grain fed to dairy calves altered the establishment and proportion of microorganisms typically found

in the rumen of adult dairy cattle. Ruminal synthesis of vitamin B_{12} was affected by lack of cobalt. *In vitro* studies indicated that rumen microorganisms require nutritional factors present in natural feedstuffs, in addition to energy, nitrogen, and minerals. Addition of rumen liquor, extracts of roughage and protein concentrate, or fermentation by-products to the *in vitro* fermentation medium increased the rate of cellulose digestion. Several laboratories have obtained a cellulolytic response with B vitamins, C_5 to C_6 fatty acids, amino acids, and partial hydrolyzates of proteins. S. Patton

546. Ruminant nutrition. Review of utilization of nonprotein nitrogen in the ruminant. W. D. GALLUP, Okla. Agr. Expt. Sta., Stillwater. J. Agr. Food Chem., 4: 625. 1956.

The utilization of nonprotein nitrogen, and the relative value of such nitrogen in meeting part of the protein requirement of ruminants, were successfully demonstrated in a series of nitrogen balance trials and practical feeding tests. Urea in amounts to supply up to 50% of the total supplemental nitrogen in fattening rations and 25% in wintering rations was efficiently utilized by beef cattle. Carbohydrate feeds were essential for efficient utilization, and methionine supplements brought further improvement. Chronic toxicity was not encountered. Acute toxicity was produced by drenching with solutions of urea and by fasting the animals before offering urea-containing feeds. S. Patton

HERD MANAGEMENT

547. Conveyor feeding system for dairy cows in stanchion and in loose housing. E. C. SCHNEIDER, Vt. Agr. Expt. Sta. Bull., 586. 1955.

A conveyor system for feeding dairy cows was designed, built, and tested in a stanchion barn. Although the system had some weaknesses as designed, 58% of the labor and 77.5% of the travel were saved by conveyor feeding as compared to hand feeding. Suggestions are made for design improvement.

A. W. Rudnick, Jr.

548. Labor requirements for dairy herds in East Texas. A. C. MAGEE, B. H. STONE, and R. H. ROGERS, Texas Agr. Expt. Sta. Progress Report, 1845. 1956.

A survey of 106 East Texas dairy farms indicated that a 16 cow herd requires 45% more handling labor per cow than does a 64 cow herd.

A. W. Rudnick, Jr.

549. Dairy pasture improvement practices and costs. A. C. MAGEE, B. H. STONE, and R. H. ROGERS, Texas Agr. Expt. Sta. Progress Report, 1839. 1956.

After a four year pasture improvement program, it has been found that "highest-yielding" improved pasture gave 770 days of additional grazing for each $100 invested. "Intermediate" and "Lowest-yielding" pastures gave 660 and 495 additional days of grazing per $100 spent on improvements. A. W. Rudnick, Jr.

550. Profitable dairy farming in Maine; its organization, costs, and returns. H. B. METCGER, Me. Agr. Expt. Sta. Bull., 542. 1955.

A study of operating costs on 152 central Maine dairy farms indicated that a profitable dairy farm in the area should meet most of the following standards: 30-35 milking cows plus replacement stock, 2.0 to 2.5 man equivalents of labor, 8,500 to 9,000 lbs. of 4.0% milk per cow, 100-110 hrs. annual labor per cow and 3 to 4 lbs. of milk per lb. of grain. Farms above av. in three of the first four factors had above av. labor incomes. Such labor income averaged $2,842 annually as compared to an over-all av. annual labor income of $949.

A. W. Rudnick, Jr.

551. Production and utilization of milk in 16 southern Illinois counties. A. REED and R. W. BARTLETT, Ill. Agr. Expt. Sta. Bull., 596. 1955.

This report summarizes a study of the past and present state of dairying in 16 southern counties in Ill. Recommendations are made for the future of dairying in the area. It is concluded that in the main, all features are favorable to an expansion of the dairy enterprise, an intensive type of agriculture, such as dairying, is necessary to most of these counties if an adequate farm income is to be realized. To reach this goal, it is recommended that there be an increase in milk production per cow, an increase in herd size, a development of Grade A markets, and an increase in production of Grade A milk. A. W. Rudnick, Jr.

552. Quality milk—does it cost more to produce? D. C. MUMFORD, Ore. Agr. Ext. Sta. Bull., 551. 1955.

A detailed cost study of 54 Oregon dairy farms indicated that essentially it cost as much to produce 100 lbs. of poor quality milk as it did to produce the same amount of Grade A milk. Although costs per cow are higher on Grade A farms, better management and adequate feeding resulted in a higher yield per cow thus lowering cost per 100 lbs. of milk.

A. W. Rudnick, Jr.

ICE CREAM

553. Manufacture of ice cream from reconstituted ingredients. G. H. NORMAN and J. P. MALKAMES, Thirteenth Air Force Central Exchange, San Francisco, Calif. Ice Cream Rev., **39**, 12: 103. 1956.

A highly satisfactory ice cream is being prepared in the Philippine Islands from anhydrous

butterfat, low heat nonfat dry milk solids, sugar, stabilizer and water. It has been found that nonfat dry milk solids can be stored satisfactorily for 6 months at temperatures ranging between 75-95° F. with relative humidities of 30-100%. Anhydrous butterfat packed in metal air tight containers is shipped and stored at 40° F. In emergencies the anhydrous butterfat can be held at 70-98° F. for as long as three months without noticeable deterioration. Corn sugar is not recommended as an ingredient because of moisture absorption and flavor deterioration during shipment. Local fruits in season such as bananas, coconuts, jack fruit chico, ates, buko, and mango have provided cheap but highly satisfactory flavors.

Acceptance of ice cream made by this procedure on the part of the Filipinos employed at various American installations indicates a large potential market for ice cream in the Philippine Islands and throughout the entire Far East. W. J. Caulfield

MILK AND CREAM

554. The problem of antibiotic detection in milk. L. A. NUTTING and F. W. BARBER, Natl. Dairy Research Lab., Inc., Oakdale, N. Y. J. Milk and Food Technol., **19**, 162. 1956.

The presence of antibiotics or other inhibitory substances in milk is a major problem. The current methods now in use are based on the inhibition of the metabolic activities of dairy streptococci in the milk as evidenced by a decrease in acid production or reductase activity. The time factor and the difficulties in attempting to integrate these procedures with daily plant operations are too great.

A rapid antibiotic detection method has been suggested. The procedure involves some type of labeling in the chemotherapeutic treatment materials prior to use. The use of a dyestuff or a long half-life radioactive indicator which would detect antibiotics in high dilution is suggested. H. H. Weiser

NUTRITIVE VALUE OF DAIRY PRODUCTS

555. Studies in vitamin metabolism. B. SURE, Ark. Agr. Expt. Sta. Bull., 565. 1956.

Several studies concerning oxygen uptake of tissues in vitamin deficiencies, vitamin interrelationships, the influence of hyperthyroidism on vitamin metabolism, and the specific action of several vitamins of the B complex on growth and economy of food utilization are reported. The section dealing with each subject is a complete formal paper. In the section on economy of food utilization it was concluded that riboflavin in cow's milk, dried skimmed milk and evaporated milk is well utilized. A. W. Rudnick, Jr.

PHYSIOLOGY AND ENDOCRINOLOGY

556. The production and composition of milk of cows cooled by shade or sprinkling during the summer months. L. L. RUSOFF, G. D. MILLER, and J. B. FRYE, JR. La. Agr. Expt. Sta. Bull., 497. 1955.

Sprinkling Jerseys or providing them shade made little difference in their milk production; however, sprinkling did increase Holstein milk production slightly. The data also indicate that sprinkling had no effect on milk composition. A. W. Rudnick, Ja.

557. Metabolism of bull semen. IV. Relationship among physical measurements, metabolic activity, and fertility. R. E. ERB, F. H. FLERCHINGER, M. H. EHLERS, and L. E. MIKOTA, Wash. Agr. Expt. Sta. Tech. Bull., 18. 1955.

Using 35 bulls of four breeds, 400 semen samples were collected and used for 16,381 inseminations. Twenty-six laboratory measurements on each semen sample were compared with non-returns, using covariance analysis. Although several metabolic tests appeared reliable for estimating fertility of bulls, the authors state that more experimental work is required before relationships between sperm concentration, motility and measures of metabolic activity can be used with maximum efficiency. A. W. Rudnick, Jr.

SANITATION AND CLEANSING

558. 3-A sanitary standards for dairy equipment. J. MARSHALL, Secy., Tech. Comm., D.I.S.A. Inc. Milk Prod. J., **47**, 6: 24. 1956.

The author discusses the 3-A sanitary standards under the following subheadings:

1. What are 3-A sanitary standards for dairy equipment?
2. How are they developed?
3. To what items of dairy equipment do 3-A sanitary standards apply today?
4. When was the program organized and what is the membership of the 3-A sanitary standards committees?

J. J. Janzen

DIFCO

Sensitivity Testing

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BACTO-SENSITIVITY DISKS

Sensitivities to the commonly employed therapeutic agents are accurately and readily determined using Bacto-Sensitivity Disks containing three concentrations of Dihydrostreptomycin, Streptomycin, Viomycin, PAS (p-Amino Salicylic Acid) and INH (Isonicotinic Acid Hydrazide). Concentrated specimen or pure cultures are evenly streaked on Bacto-Peizer TB Medium and Sensitivity Disks applied. Zones of inhibition of growth following incubation indicate if an organism is sensitive, moderately sensitive or resistant to the various chemotherapeutic agents.

Peizer TB Medium recommended for sensitivity test procedures is easily and readily prepared from Bacto-Peizer TB Medium Base and Bacto-Peizer TB Medium Enrichment (Peizer and Schecter: Am. J. Clin. Path., 20:682:1950). This is a selective medium suitable for primary culture and simultaneous sensitivity determinations of tubercle bacilli from concentrated specimen.

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Bacto-ATS Medium and Bacto-Lowenstein Medium are available in screw capped tubes with added chemotherapeutic agents. The ATS Medium is prepared according to the formula specified by the American Trudeau Society; the Lowenstein Medium according to the Jensen modification. These media are processed under carefully controlled conditions to give rapid optimal growth of the tubercle bacilli.

Bacto-ATS Sensitivity Media and Bacto-Lowenstein Sensitivity Media are supplied in sets of 12 tubes consisting of 2 tubes of control medium without therapeutic agent and one tube each of the following:

Streptomycin 10 mcg./ml.	INH 5 mcg./ml.
Streptomycin 100 mcg./ml.	Streptomycin 10 mcg. and PAS 100 mcg./ml.
Viomycin 10 mcg./ml.	Streptomycin 100 mcg. and PAS 100 mcg./ml.
Viomycin 100 mcg./ml.	Streptomycin 10 mcg. and INH 1 mcg./ml.
INH 1 mcg./ml.	Streptomycin 100 mcg. and INH 5 mcg./ml.

The above media are also supplied in packages of 12 tubes of any one medium. In addition, for more detailed determinations, ATS Sensitivity Media and Lowenstein Sensitivity Media containing Streptomycin 250 mcg., Viomycin 250 mcg., and INH 0.2 mcg. are available in packages of 12 tubes.

These media are supplied in incubator packages which permit preferred horizontal incubation.

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